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# THE INFLUENCE OF BEDDING STOCK SOURCE, PLANT BED TEMPERATURE, AND FUNGICIDE TREATMENT ON THE DEVELOPMENT OF BLACK ROT OF SWEET POTATO SPROUTS AND ON THE RESULTING CROP DURING STORAGE[1]

Robert H. Daines[2]

SUMMARY

The source of bedding stock and the temperature of sweet potato plant beds influenced the amount of black rot developing on sweet potato sprouts. At 75°F, when fleshy roots were taken from a source showing black rot or when inoculated roots were used, black rot was uncontrollable by the chemical dip treatments used. At plant-bed temperatures of 85°F and, especially, 90°F, the incidence of black rot was greatly reduced in all treatments. Under these conditions, seed-potato dip treatments reduced black rot incidence to a very low level. Of the fungicides used, the mercurials were most effective and the carbamates least effective against black rot. Of the non-mercurials, Dyrene showed the most promise for control.

INTRODUCTION.—Black rot, caused by *Ceratocystis fimbriata* (Ell. & Halst.), has long been one of the most destructive diseases of sweet potato. Heavy losses are frequent in the plant bed, in the field, in storage, and during transit and marketing.

In New Jersey, black rot was particularly destructive when manure-heated plant beds were used. Since fire- or electrically-heated beds have been in vogue, black rot has decreased in importance. However, in individual cases, even where recommended fungicidal dips have been in use, black rot has remained an important problem. This paper presents data showing that plant-bed temperature is a major factor in determining the incidence of black rot on the sprouts and on the resulting crop during storage.

REVIEW OF LITERATURE.—As early as 1926, Lauritzen (3) concluded that temperature exerts a pronounced effect on infection and subsequent development of the disease. He found that infections were most numerous at 20–23°C during an 11-day period, and that the number of infections decreased at lower or higher temperatures. At 33°C, infection did not occur. In instances where the experimental period was prolonged to 21 days the optimum temperature for disease was increased.

Poole (4) reported that naturally occurring soil temperatures affect the activities of *C. fimbriata:* "The low temperatures of the soil developing in late summer revive the activities of the fungus."

In 1951, Smith and Michael (5) and Cooley and Kushman (1) succeeded in eliminating *C. fimbriata* from sweet potato roots by subjecting them to temperatures varying from 110° to 120°F.

EXPERIMENTAL METHODS.—In an attempt to determine whether temperatures obtainable in a sweet potato plant bed would influence the occurrence of this disease, three electrically heated plant beds were set with Jersey Orange or Yellow Jersey sweet potatoes. The temperature in these beds was thermostatically controlled at 75°, 85°, and 90°F., respectively, for the entire sprout-producing period. All beds were divided into 3- or 4-sq.-ft. areas by board separators that kept treatments isolated. A light, sandy loam was used in all tests. After each experiment, the sand in the plant beds was removed and the plant bed and surrounding areas were treated with a formaldehyde drench. New sand was used for each test.

In these experiments, Jersey Orange seed potatoes selected from lots having no black rot, except where otherwise indicated, were dipped in a water suspension of spores of *C. fimbriata* previously produced on agar plates. The treated sweet potatoes were then stored 18 hours at 70–75°F and a relative humidity of 90%. The sweet potatoes were then divided into as many groups as there were treatments, and bedded after being dipped in the fungicides under test. Most tests were terminated about 6 weeks after bedding, with the examination of about 600 sprouts per treatment.

RESULTS AND DISCUSSION.—*Effect of seed source and plant-bed temperature.*—In an experiment to determine the effect of seed source and plant bed temperatures on black rot on the sprouts and the resulting stored crop, 3 sweet potato lots of the Jersey Orange variety were planted without a chemical dip treatment. In this experiment, apparently healthy sweet potato roots were selected from A) a source showing no black rot, B) a lot in which an average of 4% showed black rot, and C) roots from source A, dipped in water in which diseased sweet potatoes from source B had been mashed.

After the sprouts from these treatments (Table 1) were examined for black rot, healthy-appearing ones were planted in a sandy loam soil. At harvest, 4 bushels of sweet potatoes were stored from each treatment, and examined for black rot about 5 months later. In this experiment, both bedding stock and plant-bed temperatures were important factors in determining the amount of black rot that subsequently developed on the sprouts and on the stored sweet potatoes. At a temperature of 90°F all sprouts appeared free of black rot, regardless of source, and at 75°F considerable black rot developed on sprouts

[1] Accepted for publication November 12, 1958.
Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University, Department of Plant Pathology, New Brunswick.
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TABLE 1.—*Effect of plant-bed temperature and seed source on the incidence of black rot on sweet potato sprouts and stored sweet potatoes*

| Temperature (°F) | Source of sweet potatoes[a] | Incidence (%) of black rot | |
|---|---|---|---|
| | | Sprouts | Stored sweet potatoes |
| 90 | A | 0.0 | 0.3 |
| 85 | A | 0.0 | 1.1 |
| 75 | A | 0.0 | 1.1 |
| 90 | B | 0.0 | 0.1 |
| 85 | B | 1.0 | 4.0 |
| 75 | B | 3.9 | 5.5 |
| 90 | C | 0.0 | 2.4 |
| 85 | C | 2.6 | 5.5 |
| 75 | C | 33.6 | 7.3 |
| LSD 5% | | | 3.93 |
| LSD 1% | | | 4.00 |

[a] A, disease-free source; B, seed from source averaging 4% black rot; C, source A, dipped in water suspension of sweet potatoes taken from source B.

from both sources B and C. The black rot that developed during storage corresponded to the amount that was on the sprouts.

*Plant-bed temperatures and fungicide dips.*—The value of fungicide dips and plant-bed temperatures was tested over a 5-year period, and the data are summarized in Table 2. These data show that the amount of black rot developing on sprouts was greatest at the lowest temperature, and decreased as the temperature was increased. The percentage of diseased sprouts was significantly greater at 75°F than at either of the other temperatures. This compares with data obtained in the laboratory on the influence of temperature on mycelial growth of *C. fimbriata* (Fig. 1). Although statistical analysis did not show the 90° treatments to be significantly better than those at 85°, in all 16 cases where disease occurred over the 5 years of experimentation, the 85° treatments consistently showed more disease than did the corresponding treatment at 90°, implying significance in favor of the higher temperature.

In these tests the main effect of fungicides indicates that, even though all treatments significantly reduced disease incidence below that occurring in untreated beds, Semesan Bel (2-chloro-4-(hydroxymercuri)phenyl and isomers, 3.2%; 4-(hydroxymercuri)-2-nitrophenyl and isomers, 12%) gave significantly better disease control on the sprouts than did ferbam (ferric dimethyldithiocarbamate). At the temperature levels, 90° alone provided sufficient control of black rot to preclude significance among the treatments used. At 85°, only Semesan Bel gave highly significant control of black rot, and at 75°, all fungicides gave significant control, but no treatment reduced disease to a practical level.

In the 1958 experiments, the fungicide dip experiment was expanded to include thiram (bis(dimethylthiocarbamoyl) disulfide), Dyrene (2,4-dichloro-6-(*o*-chloroanilino) triazine), Puratized Agricultural Spray (tris(2-hydroxyethyl)(phenylmercuri)ammonium lactate), and combinations of Puratized Agricultural Spray and various fungicides, as indicated in Table 3. In this experiment the sprouts were not examined until 8 weeks after bedding, and, as expected, the plant-bed temperature again exerted a major influence on the percentage of sprouts showing the disease. At the 75° temperature, only the treatments containing mercurials provided protection against black rot; however, at this temperature the effectiveness of the mercurial seems to have been altered when used in combination with ferbam or thiram. Again at 90°, the temperature effect alone precluded significance from the use of fungicidal dips. However, since all treatments except one consistently reduced disease below that of the untreated check, it seems certain, on the basis of chance, that real significance exists in favor of most treatments tested.

In the 1958 experiment, the incidence of disease at all temperature levels was considerably greater than that reported for the five-year average. In this experiment, captan and ferbam provided no disease reduction at the 75° level, whereas a significant reduction is reported at this temperature level in the five-year study. Although no definite explanation can be given for their variations, the fact that the sprouts remained in the bed longer before pulling in 1958 than in the other years may explain the differences. Although ferbam and captan may have exerted a controlling influence in reducing the number of sprouts becoming infected from the mother sweet potato, infe tion may have been sufficiently general to allow rapid spread from sprout to sprout at this favorable temperature level. If such was the case, the beneficial

TABLE 2.—*The effect of plant-bed temperatures and seed-potato treatments on black rot development in resulting sprouts*[a]

| Fungicide treatments | Sprouts showing black rot at | | | | | | Average effect of fungicide | |
|---|---|---|---|---|---|---|---|---|
| | 75°F | | 85°F | | 90°F | | | |
| | % | Angle | % | Angle | % | Angle | % | Angle |
| Semesan Bel—1 lb. per 7½ gal. | 21.0 | 27.3 | 4.5 | 7.4 | 0.8 | 5.3 | 5.4 | 13.4 |
| Captan—1 lb. per 5 gal. | 32.1 | 34.5 | 4.7 | 12.5 | 0.9 | 5.5 | 9.0 | 17.5 |
| Ferbam—1 lb. per 5 gal. | 47.0 | 43.3 | 8.6 | 17.1 | 2.6 | 9.2 | 15.5 | 23.2 |
| Untreated check | 68.2 | 55.7 | 19.2 | 26.0 | 5.3 | 13.3 | 27.6 | 31.7 |
| Average | 40.5 | 39.5 | 7.3 | 15.7 | 2.1 | 8.3 | | |
| LSD for average effect of fungicide (angle): 5% = 4.2; 1% = 5.6 | | | | | | | | |
| LSD for average effect of temperature (angle): 5% = 9.0; 1% = 13.1 | | | | | | | | |
| LSD for fungicide at any temperature (angle): 5% = 7.2; 1% = 9.8 | | | | | | | | |

[a] Data secured over 5-year period, with years serving as replicates.

TABLE 3.—*The effect of plant-bed temperatures and seed-potato dip treatments on black rot development in resulting sprouts*[a]

| Fungicide treatments | Sprouts showing black rot at 75°F | | 85°F | | 90°F | | Average effect of fungicide | |
|---|---|---|---|---|---|---|---|---|
| | % | Angle | % | Angle | % | Angle | % | Angle |
| Untreated check | 85.1 | 67.3 | 63.4 | 50.8 | 15.0 | 22.8 | 53.5 | 47.0 |
| Thiram 1 lb. per 5 gal. | 96.1 | 78.6 | 41.2 | 39.9 | 22.6 | 28.4 | 57.0 | 49.0 |
| Ferbam 1 lb. per 5 gal. | 82.1 | 65.0 | 33.2 | 35.2 | 11.5 | 19.8 | 41.3 | 40.0 |
| Captan 1 lb. per 5 gal. | 81.9 | 64.8 | 35.4 | 36.5 | 8.9 | 17.4 | 40.6 | 39.6 |
| Dyrene 1 lb. per 5 gal. | 88.3 | 70.3 | 29.0 | 32.6 | 2.8 | 9.6 | 37.1 | 37.5 |
| Semesan Bel 1 lb. per 7½ gal. | 38.6 | 38.4 | 17.9 | 25.0 | 9.8 | 18.2 | 20.9 | 27.2 |
| Puratized Agricultural 1 pt. per 10 gal. | 29.4 | 32.8 | 8.9 | 17.4 | 2.1 | 8.3 | 11.1 | 19.5 |
| Thiram 1 lb. + Puratized Ag. 1 pt. per 10 gal. | 83.3 | 65.9 | 5.9 | 14.1 | 1.4 | 6.8 | 23.4 | 28.9 |
| Ferbam 1 lb. + Puratized Ag. 1 pt. per 10 gal. | 90.0 | 71.6 | 7.1 | 15.4 | 9.0 | 17.5 | 32.6 | 34.8 |
| Captan 1 lb. + Puratized Ag. 1 pt. per 10 gal. | 34.9 | 36.2 | 5.0 | 12.9 | 0.6 | 4.4 | 9.3 | 17.8 |
| Dyrene 1 lb. + Puratized Ag. 1 pt. per 10 gal. | 35.2 | 33.2 | 0.0 | 0.0 | 0.0 | 0.0 | 4.1 | 11.7 |
| Average for all treatments | 70.2 | 56.9 | 18.4 | 25.4 | 5.8 | 13.9 | | |

LSD for fungicide at any temperature (angle): 5% = 15.1; 1% = 20.5

[a]Year's data; angle = arcsin $\sqrt{\text{percentage}}$

effects from only mildly effective materials might be lost when sprout pulling was delayed 2 weeks, as in 1958.

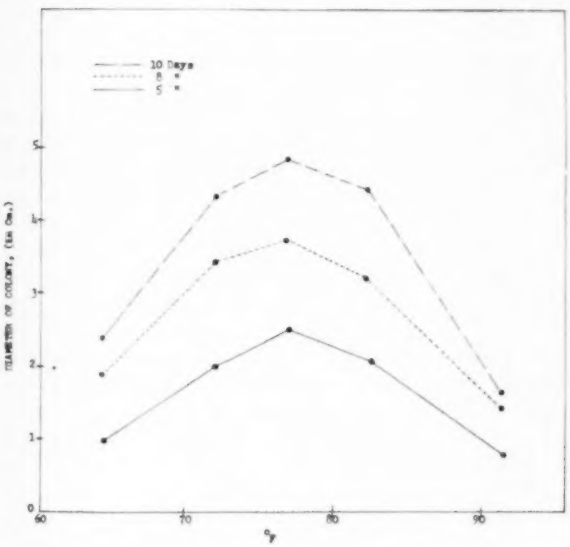


**Fig. 1.** Effect of temperature on mycelial growth of *C. fimbriata* at 5, 8, and 10 days.

In 1954, the sprouts in a bed kept at 95° were short and few in number, and emerged slowly, as compared with those grown in beds held at lower temperatures. Thus, for the control of black rot it appears that a bed temperature of 85–90°F is most desirable. However, other considerations may make 85° a better choice than 90°, since, at the higher temperature, the sprouts may be too soft to transplant well; and in addition, at 90°, Java black rot becomes a major problem (2).

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# THE EFFECT OF PLANT BED TEMPERATURE AND FUNGICIDE TREATMENTS ON THE OCCURRENCE OF JAVA BLACK ROT DISEASE OF SWEET POTATO SPROUTS[1]

Robert H. Daines[2]

SUMMARY

The temperature of the plant bed was found to influence markedly the amount of Java black rot, *Diplodia tubericola* (Ell. & Ev.) Taub., developing on sweet potato sprouts. In plant beds held at 75°, 85°, and 90°F the incidence of disease corresponded with temperature, the greatest amount at the highest temperature and the least at the lowest temperature. Mercurials were the most effective seed-potato dips, Dyrene and captan were somewhat less effective, and the carbamates showed very little activity.

INTRODUCTION.—Java black rot *(Diplodia tubericola)*, a storage disease of sweet potatoes occurring in many parts of the world, was first reported by Clendenin (1), in 1896. The literature does not cite this disease in connection with the growing sweet potato plant; in fact, Harter (3) states, "Java black rot is strictly a storage disease." The present investigation reveals, however, that, under proper conditions, Java black rot may also be a serious problem in the plant bed.

Java-black-rot-affected sprouts appear macroscopically to be identical to sprouts showing black rot caused by *Ceratocystis fimbriata* (Fig. 1). However, on examination under magnification, the long-necked perithecia distinguish the black-rot organism from the typical pycnidia of *D. tubericola*. In addition, in fresh tissue sections of the sprouts, the heavy, dark-brown mycelial strands of the Java black-rot organism are a contrast to the hyaline mycelium of *C. tubericola*.

EXPERIMENTAL METHODS.—The methods used in this study are the same as those outlined in a previous paper (2). In this investigation sweet potatoes inoculated with spores of *D. tubericola* were held for 18 hours at a temperature of 85°F and a relative humidity of 90%.

RESULTS AND DISCUSSION.—*Effect of plant bed temperatures.*—The results of a 3-year study are tabulated in Table 1. The greatest percentage of sprouts became diseased at the highest temperature, and the occurrence of disease decreased as the temperature was decreased. The percentage of diseased sprouts was significantly greater in the 90° bed than in either of the beds held at lower temperatures, and there was also more disease in the 85° bed than at the 75° temperature. This trend agrees well with laboratory studies on the effect of temperature on mycelial growth of *D. tubericola* (Fig. 2).

Daines (2) showed, in a study of the effect of temperature on black rot, that 75°F favored the disease, whereas 90° exerted a significant suppressing effect. In these same electric beds, the high temperature resulted in an increase of Java black rot, whereas disease incidence was reduced at 75°. This differential effect of temperature on the two diseases was statistically highly significant.

Results show that ferbam (ferric dimethyldithiocarbamate) is ineffective against Java black rot (Table 1). Captan (*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide), and, especially, Semesan Bel (2-chloro-4-(hydroxymercuri) phenol and isomers, 3.2%; 4-(hydroxymercuri)-2-nitrophenyl and isomers, 12%) provided significant protection against the disease. At each temperature level the mercurials showed significance over all other treatments, except at the

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Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University, Department of Plant Pathology, New Brunswick, N. J.

[2] Plant Pathologist, New Jersey Agricultural Experiment Station, New Brunswick.

TABLE 1.—*Effect of plant-bed temperatures and sweet-potato treatments on the occurrence of Java black rot on the resulting sprouts*[a]

| Fungicide treatments | Sprouts showing Java black rot | | | | | | Average effect of fungicide | |
|---|---|---|---|---|---|---|---|---|
| | 75°F | | 85°F | | 90°F | | | |
| | Angle[b] | % | Angle | % | Angle | % | Angle | % |
| Untreated check | 16.1 | 7.7 | 23.4 | 16.4 | 35.3 | 33.4 | 24.9 | 19.2 |
| Ferbam—1 lb. per 5 gal. | 14.5 | 6.3 | 25.7 | 18.8 | 33.5 | 30.4 | 24.4 | 18.5 |
| Captan—1 lb. per 5 gal. | 5.7 | 1.0 | 13.4 | 5.4 | 22.3 | 14.4 | 13.8 | 6.9 |
| Semesan Bel—1 lb. per 7½ gal. | 2.6 | 0.2 | 4.4 | 0.6 | 11.5 | 4.0 | 6.2 | 1.6 |
| Average for all treatments | 9.7 | 3.8 | 16.7 | 10.3 | 25.6 | 20.6 | | |
| LSD for fungicide (angle) at any temperature: 5% = 7.0; 1% = 9.7. | | | | | | | | |
| LSD for average effect of fungicide (angle): 5% = 4.0; 1% = 5.6. | | | | | | | | |
| LSD for average effect of temperature (angle): 5% = 10.5; 1% = 17.4. | | | | | | | | |

[a] Data secured over 3-year period, with years serving as replicates.
[b] Angle = arcsin $\sqrt{\text{percentage}}$.

TABLE 2.—*The effect of sweet-potato dip treatments and plant-bed temperatures on the development of Java black rot in the resulting sprouts*[a]

| Fungicide treatments | Sprouts showing Java black rot | | | | | | Disease average 3 temperatures | |
|---|---|---|---|---|---|---|---|---|
| | 75°F | | 85°F | | 90°F | | | |
| | Angle[b] | % | Angle | % | Angle | % | Angle | % |
| Untreated check | 22.5 | 14.7 | 28.3 | 22.5 | 48.8 | 56.6 | 33.2 | 31.3 |
| Ferbam—1 lb. per 5 gal. | 19.8 | 11.5 | 38.5 | 38.7 | 48.8 | 56.6 | 35.7 | 35.6 |
| Thiram—1 lb. per 5 gal. | 1.3 | 0.5 | 32.5 | 28.8 | 42.1 | 45.0 | 25.3 | 24.8 |
| Captan—1 lb. per 5 gal. | 1.6 | 0.8 | 20.9 | 12.7 | 36.1 | 34.7 | 19.5 | 16.1 |
| Dyrene—1 lb. per 5 gal. | 1.4 | 0.6 | 17.3 | 8.8 | 32.6 | 29.0 | 17.1 | 12.8 |
| Semesan Bel—1 lb. per 7½ gal. | 0.0 | 0.0 | 1.7 | 0.9 | 15.9 | 7.5 | 5.9 | 2.8 |
| Agricultural Puratized—1 pt. per 10 gal. | 1.4 | 0.6 | 7.5 | 1.7 | 10.6 | 3.4 | 6.5 | 1.9 |
| Average for all treatments | 6.9 | 4.2 | 21.0 | 16.3 | 33.6 | 33.0 | | |
| LSD for treatment (angle): 5% = 11.6; 1% = 16.2 | | | | | | | | |

[a] 1 year's data.
[b] Angle = $\arcsin \sqrt{\text{percentage}}$.

75° level, when significance was shown over all except the captan treatment.

In the 1958 experiments, the fungicide dip experiment was expanded to include thiram (bis(dimethylthiocarbamoyl) disulfide), Dyrene (2,4-dichloro-6-(*o*-chloroanilino) triazine), and Puratized Agricultural Spray (tris(2-hydroxyethyl) (phenylmercuri) ammonium lactate).

In this experiment (Table 2), the plant-bed temperature again exerted a major influence on the percentage of sprouts showing the disease. Captan and Dyrene provided significant and highly significant protection over the untreated check in the 90° bed; however, the two mercurials were sufficiently effective at this temperature to show significance at the 1% level over captan and Dyrene. In the 85° bed, where

**Fig. 1.** Sweet potato sprouts showing symptoms of Java black rot on lateral rootlets as well as on the main root.

the disease was much less severe, the fungicides performed much as they did in the 90° bed, i.e., the mercurials performed very well, the carbamates showed no protection, and captan and Dyrene were intermediate in effect. In the 75° bed the temperature alone did much toward controlling Java black rot. At this temperature, all treatments except check and ferbam reduced the disease to a very low level.

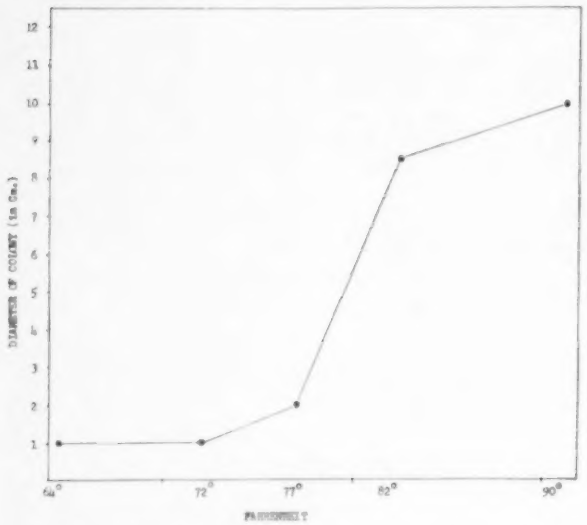


**Fig. 2.** Effect of temperature on mycelial growth of *Diplodia tubercularia* (Java black rot.)

From the data presented it is clear that the incidence of Java black rot on sprouts in the plant bed was influenced by the temperature of the plant bed and the fungicide dip treatments used.

This temperature response is undoubtedly why Java black rot is thought of as primarily a southern disease.

From the results of these studies it appears that, where Java black rot is a problem, high-temperature plant beds should be avoided.

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# RELATION BETWEEN REACTION TO RACE 15B OF STEM RUST AND REDUCING SUGARS AND SUCROSE IN WHEAT[1]

W. E. Lyles, M. C. Futrell, and I. M. Atkins[2]

SUMMARY

Reducing sugars were determined at 3 stages of growth for 4 varieties or selections of wheat: Minnesota II-50-17, Khapli, Hard Federation, and Nugget, treated and untreated with maleic hydrazide. Minn. II-50-17 and Khapli are resistant to race 15B of stem rust; the others are susceptible. Two pairs of isogenic lines, resistant and susceptible to race 15B, were analyzed for carbohydrate fractions. Reducing-sugar content was highest in resistant varieties. Untreated Minn. II-50-17 had the greatest amount of reducing sugars in all 3 stages of growth and was the most rust-resistant variety studied. Maleic hydrazide treatment significantly decreased reducing sugars in the 15B-resistant varieties and significantly increased them in the 15B-susceptible varieties, but the sugar content of the susceptible varieties did not reach the level of untreated Minn. II-50-17. Resistant isogenic lines had significantly more reducing sugar than their respective susceptible lines. Low reducing-sugar content appeared to be associated with susceptibility to race 15B of stem rust of the susceptible isogenic lines and of maleic-hydrazide-induced susceptibility of Khapli. Isogenic lines susceptible to stem rust were lower in sucrose than resistant sister lines.

INTRODUCTION.—Livingston (9), Bromfield and Peet (1), and Samborski and Shaw (11) have shown that stem-rust-resistant wheats became susceptible when treated with maleic hydrazide. Cutter (5) hypothesized that an intermediate in metabolism, possibly the phosphorylated sugars, may be required by the rust, an obligate parasite. Mains (10) and Browning (3, 4) found that carbohydrate supply influenced the rust reaction of detached leaves inoculated with rust. Shu and Ledingham (13) found that the glucose-6-phosphate oxidation pathway was actively involved in the carbohydrate metabolism of rust fungi. This investigation was made to study the relation of carbohydrate fractions to rust susceptibility in isogenic lines, 15B-resistant wheat varieties, and meleic-hydrazide-treated 15B-resistant varieties.

MATERIALS AND METHODS.—For analysis, 4 varieties or selections of wheat (Khapli C.I. 4013, Hard Federation C.I. 4980, Nugget C.I. 12620, and Minnesota selection II-50-17 C.I. 13154 of Frontana × Kenya-58 × Newthatch parentage) were grown in randomized split plots, replicated 4 times, during the 1955–56 and 1956–57 crop seasons. An aqueous 1000-ppm solution of the sodium salt of maleic hydrazide was applied at the 5-leaf stage to some of the rows at the rate of 75 ml per 10-foot row. Two pairs of "isogenic lines"

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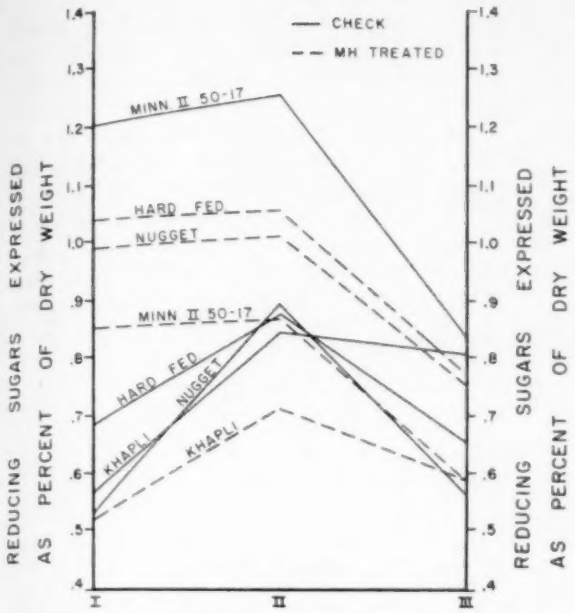


**Fig. 1.** Effect of maleic hydrazide on the reducing sugar content of race 15B stem rust resistant and susceptible wheat varieties at 3 growth stages (I, tillering; II, jointing; III, dough).

of the Newthatch-Frontana × Renacimiento-Kenya (Texas 3708-131) cross were grown in a randomized block design. These isogenic lines differed from their counterparts by at least a single gene for rust reaction.

Minnesota strain II-50-17 was resistant to many races of *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn. Khapli wheat has demonstrated resistance to a large number of races of this fungus. Nugget is highly susceptible to race 15B, but resistant to most other races common in the United States, and Hard Federation is susceptible to most races of stem rust.

Plant material to be analyzed was collected at 3 stages of growth—tillering, jointing, and soft dough. All the aboveground parts of the plants were used in the analyses. Samples were harvested late in the afternoon, weighed, dried at 65°C in a forced-draft oven, vacuum-dried to constant weight, and ground in a Wiley mill to pass an 80-mesh screen.

Labile carbohydrates were prepared for analysis by the method of Eaton and Rigler (6), and reducing sugars were determined by the semimicro method of Wildman and Hansen (14). Sucrose was determined by hydrolyzing an aliquot of the cleared solution with concentrated hydrochloric acid, determining the total sugar after inversion, and subtracting the reducing sugars. Starch was determined by combined diastatic and acid hydrolysis of the residue after extraction. The starch-free residue was then hydrolyzed by autoclaving with hydrochloric acid, and the reducing fractions determined were expressed as hemicellulose.

RESULTS.—Treatment with maleic hydrazide significantly decreased reducing sugars in 15B-resistant Khapli and Minn. II-50-17 at all stages of growth, and significantly increased reducing sugars in 15B-susceptible Hard Federation and Nugget (Fig. 1). The level of reducing sugars remained the same from the tillering to jointing stages of growth in maleic-hydrazide-treated Hard Federation, Nugget, and Minn. II-50-17, but in treated Khapli was greater at the jointing stage than at the tillering stage. The level increased sharply between tillering and jointing in untreated Khapli, Hard Federation, and Nugget, but increased only slightly in the Minn. II-50-17 selection. All treated and untreated varieties showed a decrease in reducing sugars between the jointing and dough stages of growth. Stem rust does its greatest damage during the dough stage of growth, and at this stage both Khapli and Minn. II-50-17 were significantly higher in reducing sugars than were Nugget and Hard Federation.

The decrease in reducing sugars caused by maleic hydrazide treatment of Khapli was accompanied by a marked increase in susceptibility to race 15B of stem rust. Minn. II-50-17 also increased in susceptibility,

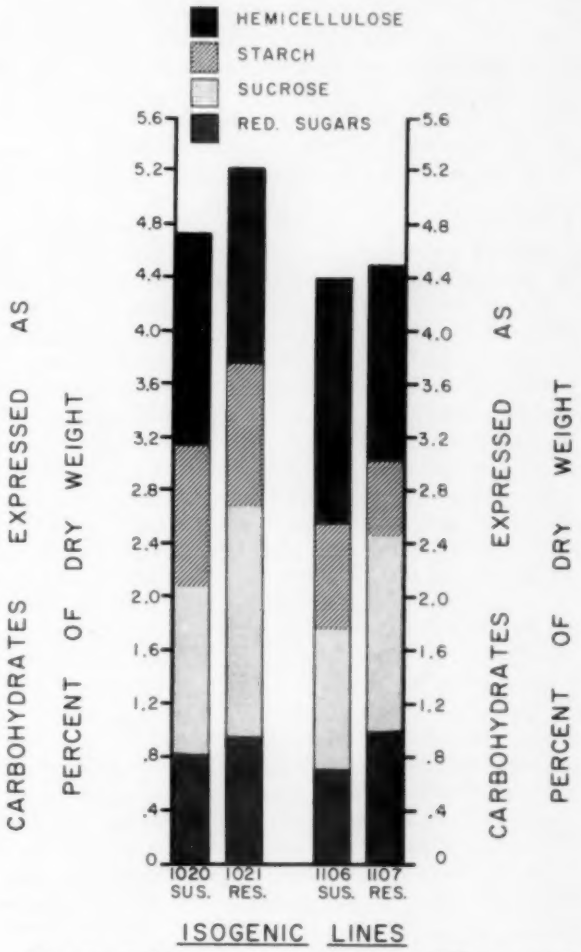


**Fig. 2.** Carbohydrate content of race 15B stem rust resistant and susceptible isogenic wheat lines at the seedling stage of growth.

but not as markedly as Khapli. The increase in reducing sugars in Nugget and Hard Federation did not change their response to stem rust.

Two pairs of isogenic lines of the Newthatch-Frontana × Renacimiento-Kenya (Texas 3708-131) cross were analyzed for carbohydrate fractions. The resistant lines carried a gene or genes for resistance to race 15B of stem rust; otherwise, the lines were very similar in genic makeup. Fig. 2 shows reducing sugars, sucrose, starch, and hemicellulose fractions for the two pairs of isogenic lines. The resistant line of both pairs of isogenic lines was significantly higher in reducing sugars and sucrose, but the comparison was not consistent for the hemicellulose fraction (Fig. 2). There was no significant difference in the starch fraction.

Discussion.—Data in this study show that the level of soluble carbohydrates, especially the reducing sugars, is much higher in 15B-resistant varieties than in susceptible ones, and that a high level of soluble carbohydrates is strongly associated with rust resistance. Resistance may not be due solely to the level of soluble carbohydrate content. Because of the complex conditions affecting it, rust resistance cannot be attributed to the effect of any single condition or class of compounds. The differences in rust reaction could have been due to varietal differences other than sugar content. However, the resistant line of each pair of isogenic lines had a higher amount of sucrose and reducing sugars than its corresponding susceptible line.

Other workers (12) showed that maleic hydrazide increased the soluble nitrogen and sucrose contents of treated Khapli plants but lowered the reducing sugars. The energy used for the hydrolysis of proteins into soluble fractions may have come from the reducing sugars. The primary synthesis of amino acids and similar compounds occurs at the expense of carbohydrates, which serve both as building material and as a source of energy. Therefore, rapid amino acid synthesis could result in a decrease in carbohydrates, as appeared in the susceptible isogenic line. Plants in which amino acid synthesis occurs slowly will often be proportionately higher in carbohydrates, as in the resistant isogenic line. Several workers (2, 7, 8) have shown that available nitrogen has an effect on the ability of the rust fungus to survive. Low levels of nitrogen lead to a higher content of soluble carbohydrates, and the development of rust (8) is retarded. Browning (3) and Mains (10) have shown that a carbon source supplied to detached leaves of a rust-resistant variety increased susceptibility to rust. Gassner and Hassebrauk (8) found that ammonium nitrogen was detrimental to leaf rust in weak light, but favorable in stronger light or when carbohydrates were fed along with ammonium salt. Again, the utilization of nitrogen and development of rust depend on the supply of a soluble carbohydrate.

The metabolic disturbances produced by maleic hydrazide in resistant varieties bear a general resemblance to those that occur at the loci of rust infections of susceptible varieties. In both cases sucrose, starch, dry weight, amino compounds, and respiration are increased. The resistant varieties made susceptible by treatment with maleic hydrazide are similar in composition to the genetically susceptible varieties.

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# INHERITANCE OF RESISTANCE OF STRAINS OF DIPLOID AND TETRAPLOID SPECIES OF OATS TO RACES OF THE CROWN RUST FUNGUS[1]

M. D. Simons, K. Sadanaga, and H. C. Murphy[2]

SUMMARY

Inheritance of resistance to *Puccinia coronata avenae* of strains of diploid *(Avena strigosa)* and tetraploid *(A. abyssinica)* oats was studied. In *A. strigosa* the light flecking reactions of C.I. 7010 to races 202, 203, 216, and 264 were all conditioned by the same single major dominant gene, and 1 minor gene probably was involved also; the highly resistant reactions of C.I. 2630 to races 205, 216, and 264 were conditioned by single dominant genes; and the light flecking reactions of C.I. 3815 to races 205, 227, and 294 were conditioned by single dominant genes. The genes for resistance carried by C.I. 7010, C.I. 2630, and C.I. 3815 were not allelic. In *A. abyssinica* the heavy flecking reactions of C.I. 7233 to races 202, 203, 205, and 264 and the 2-type reaction of this line to race 216 were all conditioned by the same single gene, which lacked dominance; and the light flecking reaction of C.I. 7232 to race 264 was conditioned by a single dominant gene. The genes for resistance carried by C.I. 7233 and C.I. 7232 were not allelic.

The resistance of Landhafer and certain other varieties of oats to all North American races of the crown rust fungus (*Puccinia coronata* Cda. var. *avenae* Fraser & Led.) discovered before 1953 led to widespread use of these varieties in oat breeding in the United States. The recent appearance of races parasitizing these varieties (5) therefore caused considerable concern, and necessitated a search for varieties resistant to these new races. Extensive testing (7) failed to reveal any hexaploid varieties with adequate seedling resistance, but a high degree of seedling resistance was found in several strains of diploid and tetraploid oats. The use of key races and subraces showed that these strains fall into groups that differ in crown rust reaction (6).

A single representative, Saia, from one of these resistant groups, has been studied genetically. In this variety some plants have been reported to carry 1, and other plants 3, dominant genes for resistance (4). Literature dealing with inheritance of reaction to the crown rust fungus has been reviewed by Finkner, Atkins, and Murphy (2). The present investigations were undertaken to ascertain the number of genes for crown rust resistance carried by certain diploid and tetraploid oats and to determine the dominance and allelic relationships of these genes.

MATERIALS AND METHODS.—Table 1 lists the strains of oats and the races of the crown rust fungus used. C.I. 7010, *Avena strigosa* Schreb., is a purified selection from C.I. 4639 (Saia). C.I. 7233, *A. abyssinica* Hochst., is a purified selection from P.I. 193958. The crown rust resistance of these two lines and of C.I. 2630 (Glabrota) and C.I. 3815 (both *A. strigosa*) has been discussed elsewhere (6). C.I. 7232 (Aberdeen 101) is a true-breeding tetraploid (*A. abyssinica*) derived from a cross between C.D. 3820 (believed to be the same as C.I. 4639) and a susceptible line of *A. abyssinica*. Under greenhouse conditions it appears to have the same reactions to races of the crown rust fungus as does C.I. 7010 (8). C.I. 7423 is a susceptible true-breeding strain of *A. abyssinica* derived from the same cross as C.I. 7232.

Methods of isolation, inoculation, and increase of cultures of *P. coronata avenae* were similar to those described by Murphy (3) and by Finkner, Atkins, and Murphy (1).

INHERITANCE OF RESISTANCE.—*C.I. 7010.*—The resistance to race 264 of C.I. 7010 appeared to be conditioned primarily by a single major dominant gene. However, data (Table 2) from crosses between C.I. 7010 and a highly susceptible variety could be explained more precisely by assuming an additional gene for crown rust reaction in C.I. 7010. The reactions conditioned by this second gene are somewhat variable, but in general it conditions a moderately susceptible, as opposed to a highly susceptible, reaction in the absence of the major gene. Under the conditions of a few of the tests, moderately susceptible plants were difficult to distinguish from highly susceptible plants; consequently, data from these tests indicated that only a single gene was involved. Plants carrying both genes may be slightly more resistant than plants with only the major gene.

The 472 $F_2$ plants that were tested for reaction to race 264 were divided into representative groups. One of these groups was tested with race 202, 1 with race 203, and 1 with race 216. In all cases the reactions of the plants to these races were similar to their respective reactions to race 264, indicating that resistance to all 4 races is controlled by the same gene or genes.

An $F_2$ progeny of a cross of C.I. 7010 with C.I. 2465 (a susceptible strain of *Avena nudibrevis* Vav.) was tested for reaction to races 264 and 216. The data substantiated the conclusions just presented with regard to C.I. 7010.

*C.I. 2630.*—$F_2$ progenies from C.I. 2630 × C.I. 3214 and C.I. 2630 × C.I. 7010 were tested with races 264

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TABLE 1.—*Average reactions*[a] *of parental strains of oats to races of P. coronata avenae used in studying inheritance of resistance*

| *Avena* species and strain number[b] | Race | | | | | | |
|---|---|---|---|---|---|---|---|
| | 202 | 203 | 205 | 216 | 227 | 264 | 294 |
| *A. strigosa* (diploid) | | | | | | | |
| C.I. 7010 (Saia) | I,0– | I,0– | 4 | I,0 | 4 | I,0– | I,0– |
| C.I. 3815 | 0– | 0– | 0– | 0– | 0– | 0– | 0– |
| C.I. 2630 (Glabrota) | I | I | I | I | I | I | 4 |
| C.I. 3214 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| *A. wiestii* (diploid) | | | | | | | |
| C.I. 1994 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| *A. nudibrevis* (diploid) | | | | | | | |
| C.I. 2465 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| *A. abyssinica* (tetraploid) | | | | | | | |
| C.I. 7233 (P.I. 193958) | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| C.D. 4549 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| C.I. 2519 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| C.I. 7232 (Abd. 101) | I,0– | I,0– | 4 | I,0– | 4 | I,0– | I,0– |
| C.I. 7423 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |

[a] I indicates no macroscopic evidence of infection; 0, flecking but no sporulation; 2, moderate resistance; 3, moderate susceptibility; and 4, high susceptibility.

[b] C.I. and P.I. refer to accession numbers of the C.R.D., ARS, U. S. D. A. C.D. refers to accession numbers of the C.C.D., E.F.S., Canadian Department of Agriculture.

and 205, respectively. The data (Table 2) indicate that the very high type of resistance of C.I. 2630 to these 2 races is governed by single, almost completely dominant genes. A single-gene hypothesis was also proposed to explain reactions to races 216 and 264 of $F_2$ and $F_3$ progenies from a cross between C.I. 2630 and C.I. 1994 (a susceptible strain of *Avena wiestii* (Steud). Whether the same or different genes condition resistance to the different races was not determined.

*C.I. 3815.*—$F_2$ progenies from crosses between C.I. 3815 and C.I. 7010 were tested for reaction to races 205 (Table 2) and 227. The data show that the resistance of C.I. 3815 to these races is conditioned by single dominant, or nearly dominant, genes. Representative $F_2$ plants were grown to maturity, and the reactions of the resulting $F_3$ populations provided no evidence against the assumption that a single gene is involved. C.I. 3815 was also crossed with C.I. 2630, and the progenies were tested with a culture of race 294 to which C.I. 2630 is susceptible. The resistance of C.I. 3815 to this potentially important race is apparently largely governed by a single major gene (Table 2). Small differences between plants of the same class, however, indicate that minor genes for resistance are also probably involved; their number was not determined. Whether the same or different major genes condition resistance to the different races was not determined.

*C.I. 7233.*—The crown-rust-resistant selection of *A. abyssinica* designated C.I. 7233 was crossed with a susceptible strain of the same species, C.I. 2519. Four $F_2$ progenies were combined and tested for reaction to race 264. The numbers of resistant, moderately resistant, and susceptible plants closely fit the 1:2:1 ratio expected under the assumption that a single gene lacking dominance was involved (Table 2). Moderately resistant plants were readily distinguishable from both homozygous-resistant and homozygous-susceptible plants. On completion of the test with race 264, the $F_2$ plants were divided into 4 representative groups for separate tests with races 202, 203, 205, and 216. Reactions were much the same as with race 264, except in plants inoculated with race 216: those resistant to race 264 were only moderately resistant to race 216, and those moderately resistant to race 264 were moderately susceptible to race 216. However, a consistent relationship of the reactions of individual

TABLE 2.—*Chi-square analyses of reactions of typical $F_2$ progenies from crosses involving crown-rust-resistant diploid and tetraploid strains of oats*

| Cross | Race | Reaction type | No. of $F_2$ plants obs. | Ratio | Chi-square probability |
|---|---|---|---|---|---|
| C.I. 3214 × C.I. 7010 | 264 | 0<br>3<br>4 | 357<br>83<br>32 | 12:3:1 | 0.8–0.7 |
| C.I. 7010 × C.I. 2630 | 205 | I,0<br>4 | 290<br>95 | 3:1 | 0.9–0.8 |
| C.I. 3214 × C.I. 2630 | 264 | I,0<br>4 | 71<br>24 | 3:1 | 0.98–0.95 |
| C.I. 7010 × C.I. 3815 | 205 | 0<br>4 | 214<br>66 | 3:1 | 0.7–0.5 |
| C.I. 2630 × C.I. 3815 | 294 | 0,2<br>4-,4 | 70<br>21 | 3:1 | 0.7–0.5 |
| C.I. 7233 × C.I. 2519 | 264 | 0<br>2<br>4 | 100<br>187<br>94 | 1:2:1 | 0.9–0.8 |
| C.I. 7423 × C.I. 7232 | 264 | I,0<br>4 | 145<br>44 | 3:1 | 0.7–0.5 |

plants to race 216 and to race 264 was obvious, and it was concluded that the same gene conditioned resistance to these 5 widely different races of the crown rust fungus. These conclusions were substantiated by data from $F_2$ progenies obtained from C.I. 7233 crossed with the susceptible C.D. 4549 and tested with races 216 and 264.

Representative $F_2$ plants from crosses of C.I. 7233 with C.I. 2519 and C.I. 7233 with C.D. 4549 were allowed to mature. The reactions of the resulting $F_3$ populations confirmed the assumption that a single gene for crown rust resistance is involved.

*C.I. 7232.*—Two different lots of $F_2$ plants resulting from the resistant derived tetraploid C.I. 7232 crossed with C.I. 7423 (a susceptible sister selection) were tested at different times for reaction to race 264. Combined data from the 2 tests (Table 2) show that the resistance of C.I. 7232 to race 264 is conditioned by a single, almost completely dominant gene. There was no evidence that minor genes were involved, as in C.I. 7010.

*Allelism of genes for resistance.*—The 3 diploid strains C.I. 7010, C.I. 2630, and C.I. 3815 were crossed in all combinations, and the resulting $F_2$ progenies were tested for reaction to race 264 to determine whether the genes for crown rust resistance carried by these strains were allelic. All 3 strains are resistant to this race. Susceptible plants appeared in all the $F_2$ progenies. The numbers of resistant and susceptible plants were difficult to fit to expected ratios, but the occurrence of susceptible plants clearly showed that the genes involved were not allelic. Results obtained by similarly testing progeny of C.I. 7232 × C.I. 7233 showed that the genes for resistance carried by these 2 tetraploid lines were not allelic.

Discussion.—The crown rust resistance found in certain strains of diploid and tetraploid oats has great potential value. However, transferring this resistance into commercially acceptable varieties of hexaploid oats will be tedious and difficult. It is hoped that the information presented here on mode of inheritance and degree of dominance or lack of dominance in some of the most promising of these sources may ease the task for oat breeders and pathologists.

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# THE RELATION BETWEEN RADIATION DOSE AND THE FREQUENCY OF MUTATIONS FOR PATHOGENICITY IN MELAMPSORA LINI[1]

E. A. Schwinghamer[2]

SUMMARY

Urediospores of *Melampsora lini* (Pers.) Lév., race 1, were irradiated with ultraviolet rays, X rays, $\gamma$ rays, and fast neutrons in a quantitative study of the frequency of induced mutations at the $A_M$ $a_M$ locus (avirulence dominant to virulence on the flax variety Dakota) for pathogenicity. Mutations at this locus were identified by their ability to infect the immune variety, Dakota. The data were fitted to a second-order polynomial to obtain estimates of the relative importance of single and multiple events in the mutation process. The mutation frequency (below the "saturation" level of treatment) was proportional to dose in ultraviolet radiation experiments, and nearly proportional to dose in neutron treatments. In X-ray or $\gamma$-ray experiments the frequency varied approximately as the square of the dose. Analogy of these results with similar analyses of chromosomes aberrations in higher plants suggests that the mutations induced by the ionizing radiations were predominantly "deletion" mutations, involving loss of a chromosome segment bearing the dominant $A_M$ allele. More localized, "point" changes are indicated by the ultraviolet data. Evidence of a "saturation" effect in the mutation rate was noted at high lethality levels for all of the radiations used. An increase in spore water content from about 45% to 70% during irradiation resulted in a sharp increase in the frequency of mutations induced by X or $\gamma$ rays. Fast neutrons were much more efficient mutagens than X rays, on the basis of equivalent energy absorption in tissue. The average maximum frequency (per cent of infections) induced by fast neutrons, X rays, and ultraviolet radiation was about 2.0, 1.5, and 0.3%, respectively. An alternative method is described for estimating the mutation rate on the basis of the total irradiated spore population.

It is well established that genetic variation in the rust fungi (9, 18, 22) may result from gene segregation and recombination, from mutation, and, as more recently demonstrated, from heterokaryosis (13). Nonetheless, little is known concerning the relative importance of these processes, particularly mutation and heterokaryosis, in contributing to the survival and pathogenic flexibility of these obligate parasites. Spontaneous mutations for spore color and abnormal development of sori on the alternate host have been clearly demonstrated, but the rare occurrence and circumstantial means of detection of spontaneous mutations for pathogenicity (3, 6, 9) indicate merely that the phenomenon probably does occur among the rust fungi.

Artificial acceleration of mutation rates to readily detectable levels is a prerequisite to a practical study of mutations concerning virulence, since analyses of such variation must be made laboriously on the host. The first reported case of induced mutations for pathogenicity in the rust fungi was that of Flor (4), who used ultraviolet radiation (UV) as a mutagen with urediospores of *Melampsora lini*. A high frequency of induced mutants in *M. lini* was subsequently obtained by Flor (5, 6) with X rays, and by Schwinghamer (16) with X rays, thermal neutrons, and ultraviolet radiation. In both instances the analyses involved heterozygous loci, where the alteration or elimination of the dominant allele (conditioning avirulence) was detected phenotypically by the expression of virulence on a previously immune host. Whereas the previous work was concerned mainly with developing procedures and comparing the mutability of several loci, the present paper summarizes experiments concerning the quantitative radiobiology of the mutation process, at a single locus. The experiments were designed to provide information on the following: 1) the practical aspects of the maximum mutation rates attainable with different radiations (differing in method of energy dissipation in tissue), and 2) the more basic aspects of the chromosomal mechanism of the mutation event, whether a mutation results from a localized genic change or from chromosomal alteration.

MATERIALS AND METHODS.—*Description of the organism.*—Despite the procedural limitations inherent in an obligately parasitic fungus, *M. lini* possesses several distinct advantages that led to its use in this quantitative study. Single genes condition pathogenicity on most of the differential host varieties, with avirulence being completely dominant. From the standpoint of the heterozygous locus and the resultant need for inducing a change in only one allele (dominant), the dikaryotic urediospore is equivalent to a haploid (haploid stage of this fungus is unsuitable for quantitative procedures). On the other hand, the "buffering" effect (23) of the second genome may be expected to increase the probability of survival of a mutant cell, particularly when the mutation is of the chromosome deletion type. Conjugate division of the dikaryophase occurs throughout the uredial stage; both the original stock cultures and mutant cultures

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are thus propagated as clones. Finally, for some loci the host infection types conditioned by the dominant and recessive alleles contrast sharply from immune to susceptible, with relatively little variation due to environment. Such highly specific responses are a prerequisite to accurate differentiation of mutants from nonmutants, especially at high-dose treatments in which some debilitated mutants may resemble normal resistant or intermediate infection types.

The test organism, obtained from Flor, was a pure uredial culture of race 1 of *M. lini*. This strain of the rust fungus can attack only 2 of the 22 flax differential varieties: Bison and Williston Brown. In studies on the genetics of pathogenicity in *M. lini* Flor (3) found that race 1 is heterozygous for avirulence (avirulence dominant) at 4 loci, with the genotypes $A_M$ $a_M$, $A_P$ $a_P$, $A_P{}^1$ $a_P{}^1$, and $A_M{}^4$ $a_M{}^4$ conditioning avirulence on the varieties Dakota, Koto, Akmolinsk, and Victory A, respectively. The latter two loci were eliminated from the comparative mutation studies because race 1 is capable of producing some infections on Akmolinsk and Victory A. Dakota and Koto are both characterized by immunity (dominant to susceptibility). The $A_M$ $a_M$ locus was selected in preference to the $A_P$ $a_P$ locus for the experiments described herein, because the relatively higher induced mutation rate for this locus (16) permitted the use of fewer plants (Dakota) for scoring mutants.

Urediospore cultures were propagated on semi-adult plants of the susceptible variety Bison. Contamination of "clean" plants (Dakota seedlings for screening of mutants and Bison plants for maintenance of pure culture) by mutant spores was kept at a minimum by standard precautions of inoculation procedure and of spatial isolation of the plants. Spores from a contaminant-free lyophilized stock culture were used to establish new cultures of fresh spores whenever any mutants were found on the unirradiated controls of an experiment.

*Radiation treatments.*—The radiation sources, methods of dosimetry, and irradiation procedures employed in these experiments have been described in a previous paper (17). The following description is therefore limited to details relevant to the data presented.

The conditions for irradiation with the X-ray source, a G. E. Maxitron 250 therapy unit, were: 250 kvp; 30 ma; filter, 1.0 mm Al; HVL 0.2 mm Cu; dose rate (in air) ranging from 700 to 1000 r/min. Gamma radiation was obtained from $Co^{60}$ sources, at the rates designated in the intensity experiments. For fast neutron treatments, spores were exposed to a $U^{235}$ source placed in the BNL reactor thermal column, at flux values ranging (between experiments) from 1.12- to $4.02\times10^8$ $N_f/cm^2/sec$. Ultraviolet (UV) radiation treatments were made in a specially designed chamber containing a number of 8-watt G. E. germicidal lamps (about 90% of UV energy emitted at 2537 A). The lamps were calibrated with an Eppley thermopile-galvanometer system and a standard lamp. The average intensity from 4 lamps was ca. 310 ergs/mm²/sec. at the spore surface (14 cm from lamps).

Spores were exposed to X rays in glass vials, to neutrons and $\gamma$ rays (intensity experiments) in plastic vials, and to UV radiation in quartz tubes (thin layer of spores on quartz slides inserted in tube). Water content of the spores was controlled by storing the vials or tubes at different relative humidity levels before irradiation; the humidity was maintained during irradiation by enclosing a capsule of the appropriate desiccant within the sealed vial or tube. The desiccant chemicals, relative humidity values, and corresponding approximate spore water content were as follows: $P_2O_5$ (dry) 0%, 20%; silica gel (dry) 5%, 25%; $NaHSO_4$ (saturated) 52%, 45%; $NH_4Cl$ (saturated) 79%, 60%; $CaSO_4$ (saturated) 98%, 70%. Throughout this paper, primary reference will be made to the per cent of relative humidity rather than spore water content.

*Inoculation procedure.*—The spores were diluted in talc, at a surviving spore-to-talc volume ratio of about 1:170, and dusted on the misted plants with a cyclone-type duster. All inoculation was done in an isolated incubation room containing moisture chambers designed to minimize the probability of contamination between chambers or treatments. Following 24 hours in the moist chambers, the inoculated plants were returned to the greenhouse. Pustule counts on Bison (survival determination) were taken about 10 days later, following primary breakthrough but preceding secondary pustule breakthrough. Mutant counts on Dakota were taken 11–12 days after inoculation.

The weight (in mg) of spores in the inoculum was increased with dose, as partial compensation for the expected lethality (17) at each dose. Weight compensation was proportionate to lethality up to the $LD_{50}$ level, but was progressively reduced (maximum of 4 times the control weight) at higher LD's because of the spore clumping problem. The resulting infection level for all experiments averaged about 70 pustules/plant at doses below the $LD_{50}$, decreasing to about 40 pustules/plant in the $LD_{50\text{-}90}$ range. At the level of potential infections scored on Dakota for a given dose, a mutant count of 100 was required to give a standard error of less than 10% of the mutation frequency. The average standard error (per cent of mutation frequency) at different doses was 14–37% in the UV experiments, 8–18% in X-ray experiments, and 6–17% in neutron experiments. Maximum error occurred at the low dose levels, where the mutation rate was at a minimum, and at the highest doses, where survival was low.

All pustule counts were made on young seedlings, on cotyledons only, for reasons of uniformity in wettability, stage of growth, and horizontal alignment (for spore deposition), as well as for simplicity in estimating the "potential" pustule survival on Dakota. Plants were rogued after emergence to avoid overlapping and disparity in size of cotyledons, and were inoculated at the time of full cotyledon enlargement. Approximately 38 pots, each containing an average of 2 Bison

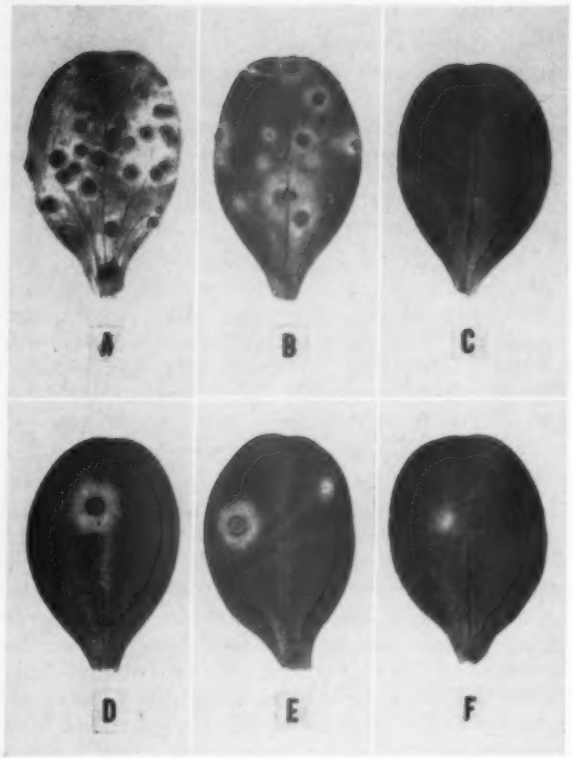


**Fig. 1.** Infections on cotyledons of flax varieties inoculated with unirradiated and irradiated urediospores of *Melampsora lini*, race 1. Survival is determined on the susceptible variety Bison; mutants are detected on the immune variety Dakota. **A)** Infection from unirradiated spores, on Bison. **B)** Infection on Bison, from spores irradiated with a 90-kr. dose of $\gamma$ rays, showing both normal and inhibited pustule types. **C)** Immune reaction on Dakota, inoculated with unirradiated spores. **DEF)** Mutant infections on Dakota, inoculated with neutron-irradiated spores; shown are normal, intermediate, and fleck types. (Note: The relative number of infections per leaf has no significance here.)

plants (location in pot marked) and 8 Dakota plants, were inoculated at each irradiation dose level. Fewer pots (average of 16) were used for the control, since this treatment was intended merely to provide an approximate estimate of percentage of lethality and of spontaneous mutations or contamination background.

*Mutation frequency determination.*—An infection, ranging from a distinct "fleck" to a fully developed pustule (type "4"), on a cotyledon of Dakota was classified as a mutant (Fig. 1). The validity of this classification and the relation of pustule vigor to severity of treatment (dose) will be discussed later. The mutation frequency ($F_I$), based on infections, was calculated by means of the relationship

$$F_I = \frac{m}{ap} \qquad 1)$$

where m = the number of mutant infections on Dakota, a = the average number of infections (survivors) per plant on Bison, and p = the number of Dakota plants screened. The term ap thus represents the estimated base of infection or potential survival on Dakota, as extrapolated from the average infection count on Bison plants exposed to the same inoculum. Formula 1 was used to determine the mutation rates of Fig. 2–5.

For an expression of the frequency in terms of urediospores (Fig. 6), the component parts of the irradiated spore population may be designated as follows: $m_s$ = mutant survivors, $m_{ns}$ = mutant nonsurvivors, $n_s$ = nonmutant survivors, $n_{ns}$ = nonmutant nonsurvivors. Here it is necessary to assume that mutant and nonmutant spores do not differ significantly in the processes (e.g., germination and appressoria formation) of host penetration, preceding the establishment of a specific nutritional or toxic host-parasite interaction. Thus, when formula 1 is expressed as

$$F_I = \frac{m_s}{n_s + m_s} \qquad 2)$$

it is apparent that $F_I$ may represent either the proportion of mutants among the infections appearing on Bison or the proportion of surviving mutant spores in the surviving spore fraction. Finally, estimation of the ratio ($F_{Sp}$) of surviving or recoverable mutants in the total spore population requires adjustment for lethality. Survival of spores can be represented as

$$\frac{n_s + m_s}{n_s + m_s + n_{ns} + m_{ns}} \qquad 3)$$

and, from the product of 2 and 3,

$$F_{Sp} = \frac{m_s}{n_s + m_s + n_{ns} + m_{ns}} \qquad 4)$$

Methods of analysis of the dose-frequency data will be described under experimental results.

RESULTS.—*Kinetics of dose-frequency relationship.*—Apparent curvilinearity in the hand-fitted dose-frequency curves for X-ray treatments was confirmed in preliminary fitting of the data to the equation $F = ax^b$ (where F = mutation frequency and x = dose), with the derived values of $b$ ranging from 1 to 2 (average = 1.7 for 3 experiments). A value of $b = 2$, indicating that mutation frequency varied as the square of the dose, would imply that a mutation results from a 2-hit *or* 2-target chromosomal event (1, 11). If one assumes that the mutations may be "loss" mutations—involving the loss of a chromosome portion bearing the $A_M$ gene—it is probable that such deletions may result from a variety of chromosomal rearrangements involving both single and multiple breaks (1, 7, 10). The above estimates of $b$, therefore, are meaningful only in indicating the existence of a predominantly second-order type of mutation kinetics, but do not distinguish the single events from the multiple events. In view of this, the final dose-frequency analyses were made by fitting the data, by the method of least squares, to the polynomial

$$F = bx + cx^2 \qquad 5)$$

In the application of this equation the curve was forced through the origin, since the mutation frequency at dose zero (spontaneous mutations plus contaminants) in most experiments approached the zero level of frequency. Estimates of the parameters $b$ and $c$ obtained for each experiment were used to define the magnitude of the linear and quadratic effect, respectively, in contributing to the mutation event.

Plots of radiation dose versus mutation (avirulence to virulence) frequency for the $A_M$ $a_M$ locus are given in Fig. 2–5, and the corresponding estimates of parameters for the curves are presented in Table 1. The mutation frequencies ($F_1$) are based on the infection (survival) values obtained at each dose, as indicated by formula 1. The survival curves of Fig.

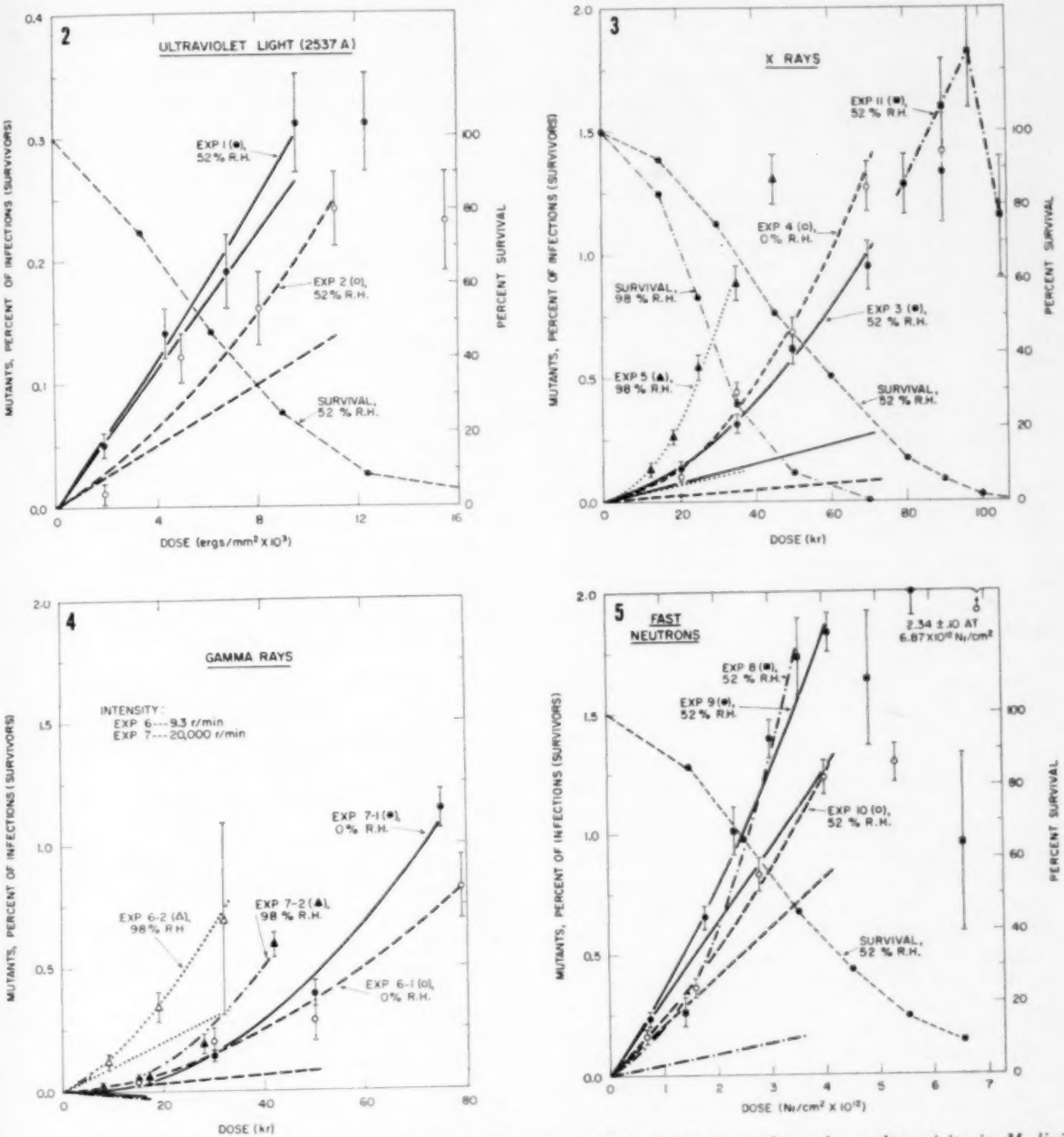


**Fig. 2-5.** Effect of radiation dose on the mutation frequency ($F_1$) of the $A_M$ $a_M$ locus for pathogenicity in *M. lini*. The experimental points (±S.E.) are fitted to the polynomial $F = bx + cx^2$, by the method of least squares. The straight lines represent the linear component for the corresponding identified curves. Relative humidity percentages denote the humidity level at which the spores were maintained during irradiation. Survival (per cent of control) curves represent average values derived from earlier dose-survival data; survival in γ-ray treatments, not illustrated here, approximates that of X-ray treatments. Experiment 11, involving only high dose treatments, is plotted to illustrate a "saturation" effect.

2–5, derived from earlier dose-survival experiments (17), were used to estimate the "expected lethality" and the compensation in spore weight needed for the inoculum at each dose. Unfortunately, the use of relatively fewer Bison plants for the unirradiated controls did not warrant the derivation of similar curves for "actual survival" from the mutation experiment data.

It is important to note that doses giving more than 80% lethality were not included in the curve-fitting computations, because of the variability or "leveling-off" in mutation frequency that occurred with all of the radiations at some point above this level of treatment. Exclusion of these points permits a more reasonable evaluation of some consistent trends or processes underlying the mutagenic action of each radiation. The "saturation" effect (mutation frequency, based on survivors, reaches a peak that may or may not be followed by an actual decline) is a fairly common phenomenon for irradiated fungi (14), particularly in UV treatments. Although some of the fluctuation of the points at the high doses in Fig. 2–5 is obviously attributable to the procedural factor of relatively low survival, as indicated by the error limits for these points, it is recalled that the absolute survival and yield of mutants was substantially increased by partial compensation (use of more spores with increasing dose) for lethality. It is considered probable that part of the variability has a biological basis in the sense that additional cellular systems (some of them selectively affecting either mutation or lethality) may become radiosensitive at higher doses; complex interaction of this type would almost certainly be more sensitive to environmental and procedural variables in the experiment. The data for the $A_M$ $a_M$ locus do not warrant an accurate estimate of the dose range in which the mutation frequency peak occurs for each radiation, but varying degrees of leveling-off were noted in most of the experiments (including those not plotted in Fig. 2–5), and it is suggested that saturation probably does occur, at some dose above the $LD_{80}$. Attention is directed here to the saturation effect simply because 1) it forms the basis for determining the data that can reasonably be excluded from the curve-fitting analyses, and 2) it is a practical consideration relating to the maximum yield and most efficient induction of mutants.

*Ultraviolet radiation treatments.*—It is evident from the curves of Fig. 2 and their constants (Table 1) that the frequency of UV-induced mutation is directly proportional to the dose, up to a dose level of about 10,000 ergs/mm$^2$ ($LD_{70-80}$). Linearity was also apparent in a preliminary experiment (not shown here), in which the spores were irradiated at 98% relative humidity.

*X-ray and γ-ray treatments.*—Some linear effect is apparent in the curve of Experiment 3, but the relation between mutation frequency and X-ray dose (Fig. 3; Table 1) is essentially nonlinear, irrespective of the spore water content during irradiation. In comparison with the UV radiation results, the saturation phenomenon in X-ray treatments apparently occurs only at noticeably higher lethality levels. A slight but consistent decline in the rate of increase was noted at the maximum dose ($LD_{90}$) in most X-ray experiments. In Experiment 11, where the dose range was concentrated at high lethality levels, the peak mutation rate occurred at about $LD_{97}$.

In the γ-ray experiments the dose range was concentrated at the lower lethality levels to permit the derivation of a more reliable estimate of the linear component. As noted for X rays, the effect of γ-ray dose on mutation frequency (Fig. 4) is predominantly curvilinear (quadratic). Here also, this general relationship appears to be largely independent of spore water content or of radiation intensity, as discussed later. The large linear component of Experiment 6-2 is questionable; no infection occurred at the 46 kr. dose, and survival at 32 kr. was so low that few mutants were recovered, as reflected by the large standard error. It is of interest that mutation rates shown for γ-ray experiments at 0% relative humidity appear significantly lower than the rates for the comparable X-ray experiments at 0 and 52% relative humidity. This would not be unexpected, on the basis of linear energy transfer (LET) differences between the 2 radiations, but the data do not warrant a definite conclusion on the comparative mutagenic effectiveness. In a preliminary γ-ray experiment, involving only 4 doses at each of 3 intensities, the mutation rates approximated those of the X-ray experiments. This equivalence in rate could have been due to more densely ionizing secondary emissions from the glass vials in which the spores were γ-irradiated, whereas only plastic vials were used in the γ-ray experiments of Fig. 4.

*Fast neutron treatments.*—The dose-frequency relationship in fast neutron treatments is almost the reverse of the analysis presented for X rays. Both linear and nonlinear components are significant, but the former is clearly predominant, as shown by the analyses of Experiments 9 and 10 (Fig. 5). The decided nonlinear nature of the curve for Experiment 8 is questionable. Only 3 points were used in fitting the curve, and curvilinearity is exaggerated by the effect of the weighting method on the low frequency at the lowest dose. The very high mutation rate at the highest dose in Experiment 10 is the maximum rate recorded for any experiment. It is considered unlikely that the higher reactor flux for this experiment would have been a contributing factor in the lack of a levelling-off effect.

*Relative total number of mutants induced by UV rays, X rays, and fast neutrons.*—A measure of the relative biological effectiveness (RBE; see Zirkle, 23) of the ionizing radiations in inducing mutants necessitates a comparison of mutation rates at a given dose level, dose here being expressed in terms of rads (rad = 100 ergs/gm of tissue). Such a comparison of the mutation frequency induced by fast neutrons,

TABLE 1.—*Statistics of the relation between radiation dose and the frequency of mutation of the $A_{M}a_{M}$ locus for pathogenicity in Melampsora lini*

| Expt. no.[a] | Radiation | Relative humidity of spores (%) | Parameter estimates of equation[b] $F = bx + cx^2$ $b \pm$ S.E. | $c \pm$ S.E. | Goodness of fit[c] | Significant component[d] of dose-frequency curve: linear (bx) or quadratic ($cx^2$) |
|---|---|---|---|---|---|---|
| 1 | Ultraviolet | 52 | 2.74 ± .55 | 0.04 ± .08 | Good | Linear |
| 2 | Ultraviolet | 52 | 1.23 ± .49 | 0.09 ± .06 | Poor | Linear |
| 3 | X rays | 52 | 0.386 ± .180 | 0.015 ± .004 | Good | Linear and quadratic |
| 4 | X rays | 0 | 0.114 ± .141 | 0.026 ± .003 | Good | Quadratic |
| 5 | X rays | 98 | 0.338 ± .302 | 0.065 ± .012 | Good | Quadratic |
| 6-1 | Gamma rays | 0 | 0.156 ± .149 | 0.011 ± .003 | Good | Quadratic |
| 6-2 | Gamma rays | 98 | 0.990 ± .590 | 0.041 ± .036 | Good | (?)[e] |
| 7-1 | Gamma rays | 0 | −0.137 ± .108 | 0.021 ± .002 | Good | Quadratic |
| 7-2 | Gamma rays | 98 | −0.152 ± .130 | 0.035 ± .005 | Good | Quadratic |
| 8 | Fast neutrons | 52 | 4.5 ± 7.3 | 13.0 ± 2.9 | Poor | (?) |
| 9 | Fast neutrons | 52 | 31.6 ± 3.6 | 3.5 ± 1.1 | Good | Linear and quadratic |
| 10 | Fast neutrons | 52 | 20.4 ± 3.3 | 2.7 ± 1.1 | Good | Linear and quadratic |

[a] The 2 parts of experiment 6 and 7 were done simultaneously. All other experiments were done at separate times, and measures of error between such experiments are not available.

[b] Experimental data fitted to this equation by the method of least squares. S.E. = standard error of the given estimate. Comparisons of the *b* and *c* values between experiments are valid for a given radiation; similar comparisons between experiments involving different radiations are not valid, since the magnitude of the values depends on the dose units used.

[c] A good fit implies chi-square nonsignificance at the 5% level of probability; a poor fit denotes significance.

[d] Where both the linear and quadratic estimates of the mutation frequency are significant, the predominant component is underscored.

[e] See text for discussion of questionable significance in Experiments 6-2 and 8.

relative to that induced by X rays (52% relative humidity), gives RBE values ranging from about 22 (at a mutation frequency of 0.10%) to 14 (at a mutation frequency of 1.5%). With respect to X-ray treatments at 98% relative humidity the corresponding range is about 14 to 8. The near-linear dose-frequency curve for neutrons and the curvilinear relationship for X rays results in a "sliding" RBE. Such a unit is of limited meaning, and is mentioned here only to give an estimate of the *range* of relative effectiveness, which may aid in interpreting the physical nature of the mutation process.

In Fig. 6 the mutagenic effectiveness of ionizing and non-ionizing radiations is compared on the common denominator of lethality. Expression of mutation frequency ($F_{Sp}$) here in terms of the irradiated spores, rather than infections, provides a means of evaluating treatment conditions that would provide the maximum percentage of recoverable mutants. The total *number* of mutants recovered from a given quantity of irradiated spores would in turn depend on the efficiency of the inoculation procedure and on the resulting level of infection. The ratio of the maximum mutant "yields" induced by fast neutron, X-ray, and UV treatment is approximately 7:3.5:1. A comparison of the average maximum mutation frequency ($F_I$) for the same radiations in Fig. 2–5 gives a somewhat different ratio, of roughly 7:5:1, corresponding to frequencies of 2.0, 1.5, and 0.3%. The curves in Fig. 6 peak at about 50–55% lethality, but it is recalled that this is on a basis of *expected* lethality. If the "actual lethality" values of each experiment had been employed, the peaks would have occurred in the 55–65% lethality range. This applied particularly to the neutron data, since spore sensitivity to neutrons in the mutation experiments (actual lethality) appeared significantly greater than predicted on the basis of the earlier dose-survival curves. The maximum frequency for the neutron experiments (Fig. 6) is thus slightly exaggerated, and it is suggested that

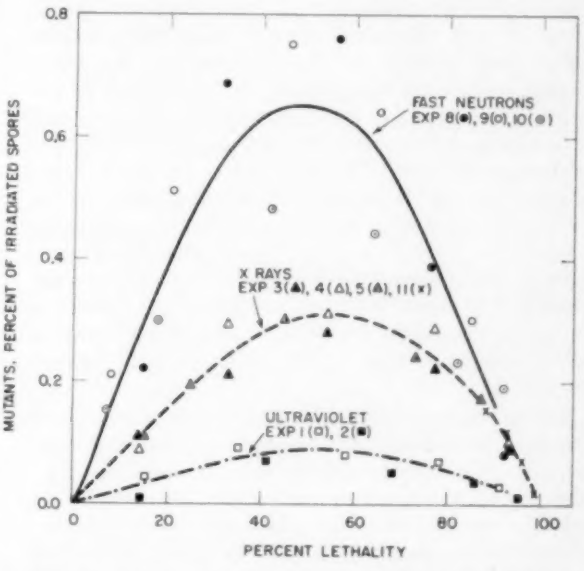


**Fig. 6.** Comparative mutation rates for ultraviolet-ray, X-ray, and fast-neutron treatments, expressed on the common basis of lethality. Mutation rates ($F_{Sp}$) and lethality estimates are derived from the data of Fig. 2–5 (see text for derivation). Each curve represents average values for the designated experiments. Gamma-ray experiments are omitted because the doses used were too low to indicate the frequency "peak."

the 7:5:1 ratio of maxima is the more reliable estimate of relative efficiency. Concerning the total number of mutants ($A_M$ $a_M$ locus) induced by irradiation in all experiments, more than 13,500 mutants were scored in an estimated $2.9\times10^6$ screened infections.

The ordinate ($F_{sp}$) may also be plotted against dose, rather than lethality, for comparisons within a given radiation treatment. In either case, however, an estimate of $F_{sp}$ involves the assumption of equal capability for host penetration among mutant and nonmutant spores, as described earlier. Expression of mutation frequency ($F_I$) in terms of infections is not subject to this restriction and does not require an estimate of the *percentage* of survival at each dose. It is therefore a more valid unit for derivation of fundamental information concerning the mutation mechanism.

*Factors that may modify the mutation frequency.*—1) *Radiation intensity.*—A comparison of dose-frequency curves (52% relative humidity, 4 doses per curve) at 3 widely differing intensities was made in a preliminary $\gamma$-ray experiment, referred to earlier. The curves for the 20,000 r/min. and the 4167 r/min. treatments were almost identical; at 56 r/min. the mutation frequency was lower at all 4 dose levels, but the difference barely exceeded the standard error. In the experiments of Fig. 4, only the 2 extreme intensities, 9.3 r/min. and 20,000 r/min., were used. Here the results were conflicting; a slight increase in frequency occurred at the high intensity with spores irradiated at 0% relative humidity, whereas the low intensity was much more effective at 98% relative humidity. The relatively low frequencies of Experiment 7-2 may reflect an effect of lower water content rather than of intensity. Lack of evidence for a consistent intensity effect under the conditions of the experiments does not rule out such a phenomenon. If chromosome breaks are involved in the mutations, it is recognized that the appearance of an intensity effect at the lowest intensity used is dependent on the "life" of the breaks. The use of still lower intensities (1 r/min. or less) appears necessary, but the resultant long exposures (ranging from days to weeks) needed with this organism complicate problems of control over some experimental variables, such as spore viability during treatment at the high humidities.

2) *Spore water content.*—Tissue water content is the most sensitive variable studied. Spore sensitivity, as reported earlier (17), is sharply increased at a high water content in treatments with UV rays, X rays, and $\gamma$ rays. Similarly, the mutation frequency at a given dose is roughly doubled in X-ray treatments (Fig. 3). No significant change in frequency is noted at relative humidity levels of 52% or less. In $\gamma$-ray treatments (Fig. 4) the frequency is increased by a factor of 4 in Experiment 6, and by a factor of about 2 in Experiment 7. In the latter experiment the effect is possibly not at a maximum, because the doses at the high intensity were applied in a short period and the spores had not been kept at 98% relative humidity long enough before irradiation to reach the maximum water content (ca. 70%). No definite effect of moisture on spore sensitivity was noted in a preliminary UV experiment (98% relative humidity), but this requires confirmation.

3) *Age and cultural background of spores.*—An experiment comparing the mutation frequency of spores stored in the refrigerator at 40°F for 2 weeks, 2 months, 3 months, and 8 months (lyophilized) revealed no statistically significant difference when irradiated with X rays at a single dose of 60 kr. Likewise, there was no significant difference in mutation frequency in an X-ray experiment comparing spores grown on a susceptible (Bison) and a semi-resistant (Ward) variety of flax. A third X-ray experiment, in which the irradiated spores were stored for 1, 2, and 3 days before inoculation of the plants, gave no conclusive evidence of a change in frequency. It is considered unlikely, however, that a post-irradiation storage effect would not exist under certain conditions of spore moisture content, temperature, or other variables (14).

*Miscellaneous (procedural) factors.*—The practice of using proportionately more spores to compensate for lethality at increasing doses of radiation is not subject to the spore clumping problem at the spore compensation ratios used in these experiments. This was ascertained in an X-ray reconstruction experiment in which the spore weights in the spore-talc mixture were 12 mg for the control and 25 mg for the 60-kr. ($LD_{65}$) treatment. A third sample contained a mixture of 25 mg of spores irradiated at 60 kr. plus 25 mg of spores irradiated separately at 120 kr. ($LD_{99}$). All 3 samples thus contained roughly the same weight of surviving spores, but varying amounts of inactivated spores. The resulting mutation frequencies ($F_I$) for the 60-kr. and 60+120-kr. treatments were 0.99% and 0.90%, respectively, the difference being nonsignificant. The mixed sample simulated a 4:1 compensation for lethality, the maximum ratio employed in mutation experiments at high dose levels. Thus there appeared to be little distortion in mutation rate due to spore clumping, or to possible competition for stomatal entrance by germ tubes from the high proportion of spores inactivated (death of spores exposed to ionizing radiations occurs *after* germination) by the radiation treatment (17).

In treatments with ionizing radiations (to a lesser degree with UV radiation), increasing injury to surviving spores at increasing doses resulted in a progressively higher proportion of weak or nonvigorous infections (Fig. 1). Mutants from 3 X-ray experiments were classified into 2 arbitrary groups: 1) *normal*, including full-sized, sporulating pustules, and 2) *inhibited*, including "flecks" and slowly developing or weakly sporulating pustules. The prevalence of normal infections at doses of 30, 60, and 90 kr. was 72, 55, and 45% (mean deviation between experiments at each dose was ca. 5%), respectively. In 19 cultures

grown (on Dakota) from weak pustules (flecks that eventually produced some spores), 10 appeared normal in the second uredial generation, 5 were classified as doubtful, and 4 remained inhibited. Sporulation appeared to be restricted more than mycelial growth. Apparently some degree of genetic damage is incurred by the severely inhibited types. This inhibition of pustule development occurs among both mutant and nonmutant infections. In view of the delayed appearance of the flecks at the high doses, it was apparent in the first X-ray experiment that premature counts on the susceptible Bison plants could result in an underestimation of the potential survival on the immune variety Dakota. Since mutant counts on Dakota were made later, with a greater degree of accuracy, the discrepancy (gain) in frequency would be greatest at the highest lethality levels, thus erroneously accenting the curvilinearity. Infection (survival) counts on Bison plants inoculated with spores irradiated at high doses were therefore taken at least 1 day later than corresponding counts for the control or low-dose treatments.

Differences in average area of cotyledons of the variety used to estimate survival, relative to the area of the variety used for mutant detection, require the application of a correction factor proportionate to the area difference. This would apply particularly in studies of relative mutation rates of several loci, each locus requiring a different host variety for detection of mutants. Corrections may be based on planimeter measurements of cotyledon area, or on comparative average infection counts on cotyledons of susceptible and immune varieties inoculated with mutant strains that can attack both. The latter adjustment would take into consideration not only differences in area but also other physical or morphological characteristics, such as the number of stomata per unit area. An "isogenic" line (see Flor, 3) Bison$^{6}$ × Dakota, was used as the mutant detector in the most recent experiments described herein. Use of such lines, when available, provides perhaps the most satisfactory approach to the problem of estimating potential infection on an immune detector variety.

Discussion.—Use of the term "mutation" to define the induced change in pathogenicity in *M. lini* is admittedly restricted to phenotypic criteria, and it is recognized that conclusive evidence for a true mutational event must await inheritance tests. In lieu of the latter, however, strong evidence for a mutation is offered by the fact that the induced change from avirulence to full virulence duplicated the phenotype known to be expressed by the $A_M\ a_M$ and $a_M\ a_M$ genotypes, respectively (3). It is also noted that, of the mutant cultures propagated for several generations, all retained their virulence on the previously immune host variety (5, 6, 16).

In attempting to gain some understanding of the mutation mechanism (1, 2, 10, 11, 12) it is necessary to determine, as far as the data permit, whether the mutation represents an intragenic (point mutation) change or an intergenic (chromosomal) change. Although no cytological or genetic studies of the mutants have been made, it is possible to establish a tentative hypothesis by a comparison of the mutation dose-frequency curves with the dose-frequency curves for chromosomal aberrations in higher plants (1, 7, 10, 21). Some general conclusions for higher plants (mainly Tradescantia and maize) of interest in such a comparison are the following:

a) The yield of simple (chromosome, chromatid, or isochromatid) breaks is proportional (linear) to the dose of X rays, $\gamma$ rays, and neutrons. The densely ionizing radiations, e. g., fast neutrons, are more efficient than the more sparsely ionizing X rays or $\gamma$ rays. UV radiation produces chromatid breaks (also proportionate to dose) but relatively few isochromatid or chromosome breaks.

b) Structural changes involving exchanges between 2 or more breaks increase more rapidly than the first power of the dose (approaching the square of the dose at a high intensity) in X-ray and $\gamma$-ray treatments. In view of the spatial dispersion of the breaks, the opportunity for exchange ("concentration" of breaks) is thus time-dependent and dose-dependent. Neutron-induced exchanges vary linearly with dose, presumably because both breaks participating in an exchange are produced by a single, densely ionizing proton. UV radiation produces mainly terminal deletions (from chromatid breaks), which vary directly with dose. In general, the prevalence of complex, 2-hit aberrations induced by UV treatment is much lower than in treatments with ionizing radiations, due partly to a lower breakage efficiency and/or a lower frequency of union between breaks.

c) A high proportion of mutations induced by ionizing radiations is ascribed to losses of small sections (interstitial deletions) of chromosome material following chromosome breakage. This implies a probable similarity between the dose-frequency curves for induced mutations and for 2-hit aberrations. Mutations induced by UV radiation have most frequently been characterized as "point" changes or simple deletions, specifically terminal deletions, although recent work with maize endosperm markers (2) indicates a higher prevalence of loss mutations (including interstitial deletions) than heretofore realized. The dose-frequency response for UV radiation is usually linear. The proportion of complex aberrations or degree of sterility at doses giving equivalent mutation rates is lower for UV radiation than for ionizing radiations, again suggesting subtle point changes.

The dose-frequency kinetics for mutations (*below* the $LD_{s0}$ level of treatment, as already described) at the $A_M\ a_M$ locus in *M. lini* are analogous to the above-mentioned criteria in the following respects: a) linearity in UV experiments; b) curvilinearity in X- and $\gamma$-ray experiments, with the frequency varying approximately as the square of the dose; c) approximate linearity in neutron experiments, part of the quadratic component being attributable to $\gamma$-ray con-

tamination; d) a UV-induced mutation frequency relatively lower than that induced by ionizing radiations, and a higher efficiency of neutrons as compared to X or $\gamma$ rays; e) a lower degree of associated injury, especially at high doses, in UV-induced mutants than in mutants induced by the ionizing radiations. It is therefore postulated that the mutations to virulence at the $A_M$ $a_M$ locus (and probably at other loci) induced in *M. lini* by X rays, $\gamma$ rays, and fast neutrons result mainly from loss of chromosomal segments bearing the dominant $A_M$ allele. Such deficiencies could result from loss of acentric fragments by simple deletion or by exchanges between chromosome arms. Interchanges between homologous chromosomes would be excluded by the dikaryotic condition of the uredial stage. Aside from physiological injury due to irradiation, survival of the mutant spore would depend on the "size" of the deletion (loss of many minor genes or of a few genes vital to survival) containing the $A_M$ allele, and on the number of other deletions the spore may carry. Apparently new, intermediate-reaction phenotypes (high dose effect, Fig. 1), not necessarily involving any of the known pathogenicity loci, could result from such concomitant deletions of unrelated but "critical" genes. In the above interpretation of a predominantly 2-break process it is borne in mind that a minor portion of the mutations may result from single-break deletions or from rearrangements involving more than 2 breaks. Also to be considered is the lesser possibility that some of the mutants, especially the slightly inhibited types, may represent position effects due to inversion or translocation of breaks involving the $A_M$ allele. Absence of a definite intensity effect in $\gamma$-ray experiments deprives the above interpretations of further confirmation but, as described earlier, detection of such an effect requires more exhaustive tests.

The results for UV-induced mutations suggest less drastic "point" changes, although not necessarily on the genic or chemical level. The linear dose-frequency response does not exclude one-hit aberrations like terminal deletions (or very small interstitial deletions). As with higher plants it is possible that breaks may be prevalent but restitute preferentially, and thus do not contribute to mutation. It is considered probable that the frequency of mutations induced by ionizing radiations, as compared to UV treatment, would be relatively lower in the haploid stage of this organism, because of an inviability of a major fraction of the "loss" mutants. In this fungus, however, quantitative work with the haploid spores is not feasible.

Comparison of the dose-frequency data with those reported for other fungi, rather than higher plants, is less meaningful, because of the lack of cytological evidence in fungi. The frequency of reports of linear dose-frequency responses to X-ray treatments in fungi does not necessarily preclude mutation-by-breakage phenomena. Haploidy in the test organism and the common practice of plotting the sum frequency of more than 1 class of mutants, particularly morphological mutants (as opposed to more definable biochemical variants), tend to obscure nonlinearity and other criteria for involvement of extragenic processes. Nevertheless, evidence for the latter has been presented for a number of fungi. Sansome, Demerec, and Hollaender (15) noted an increase in the rate of morphological mutants *(Neurospora crassa)* with an increase of X-ray intensity. The ratio of partial sterility in the mutant versus the nonmutant classes was higher for X rays than for UV treatment. Swanson, Hollaender, and Kaufmann (20) increased the X-ray-induced (but not the UV-induced) frequency of morphological mutants in *Aspergillus terreus* by pretreatment with infrared rays, and attributed this effect to enhancement of chromosome rearrangements. Stapleton, Hollaender, and Martin (19) compared the mutagenic efficiency of X rays and $\alpha$ particles in the same organism and concluded that both genic and extragenic mechanisms contributed to the production of viable mutations. In general, it appears probable that the mechanism of induced mutations for pathogenicity in *M. lini* is not unlike that of mutants for cultural characters in other fungi or for a variety of characters in higher plants. The relative importance of genic versus deficiency-type mutations is obviously dependent on a host of factors, including the type of mutagen, the nature of the gene or its location on the chromosome, and the cytologic state of the organism.

Knowledge concerning the induced mutability of different genes for pathogenicity should prove useful to the plant breeder in attempting to evaluate the usefulness of corresponding genes for resistance in the host. Such practical implications of the mutation studies have been discussed by Flor (3, 6). Whether induced rate spectra are indicative of a similar pattern for spontaneous mutations in nature, remains to be determined. The combined radiation level to which the fungus is exposed in nature—including UV radiation, terrestrial gamma rays, and emissions from radioactive elements absorbed by the host plant—probably contributes significantly to the spontaneous rate. Urediospores, in particular, are vulnerable to the mutagenic UV spectrum of direct sunlight. It is considered likely, however, that chemical mutagenesis may be the major contributing factor in spontaneous mutation. Potentially mutagenic chemicals, both metabolic analogs and toxic agents (produced by host or pathogen as a result of impaired metabolism), may well occur in necrotic or senescent host tissue under a variety of circumstances. The sudden occurrence of new, virulent races of the rust fungi has often been attributed to a selective increase of previously existing races on newly introduced resistant host varieties. Attention should also be given to the possibility that semi-resistant or resistant (assuming some sporulation) plants may contribute to the *origin* of new races by applying "mutation pressure" as well as "selection pressure" on the pathogen. On susceptible plants ("harmonious" host-parasite interaction)

the chemical mutagenic factor would be expected to be of less importance, but the higher rate of spore production would increase the probability of mutations due to solar radiation or to inherent thermal instability of certain genes. Much research on these and related problems is needed to clarify the relation of environment to spontaneous mutation in plant pathogens.

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# A METHOD OF ASSAYING SOILS FOR NUMBERS OF ACTINOMYCETES ANTAGONISTIC TO FUNGAL PATHOGENS[1]

Leonard J. Herr[2]

SUMMARY

A triple-agar-layer plate technique, developed for isolating soil actinomycetes antagonistic to plant-pathogenic fungi, has been used to assay for antagonists of *Fusarium roseum*, *Rhizoctonia solani*, *Pythium ultimum*, and *Verticillium albo-atrum*, and undoubtedly can be used with other fungal pathogens. The 3 layers are: 1) a sterile-water agar layer, 2) soil-suspension agar layer, and 3) assay-fungus agar layer. Statistical analyses of the method showed that 10 plate sets per soil sample were satisfactory and that distribution of counts of antagonists was not normal. It was concluded that non-normality had little effect on analysis of variance of this type of data.

Many of the techniques now used for isolating actinomycetes antagonistic to fungi were designed primarily for isolating antibiotic-producing organisms, and are not satisfactory for quantitative determinations of antagonists in soils. Meredith (4), Cooper and Chilton (2), and others have studied the distribution of antagonists in soils by isolating the actinomycetes on soil-dilution plates and then streaking all or randomly chosen isolates with the assay fungal pathogens. This method was rejected as too time-consuming. Freeman and Tims (3) employed a dual-layer-agar plate technique in which antagonism is assayed directly on the soil-dilution isolation plates, eliminating any need of transferring isolates for streaking tests. This general method tests all actinomycetes isolated for antagonism, and is rapid and less laborious than the streaking technique. A modified version of their technique was successful in a number of small experiments, but, with certain soil samples, assay plates were consistently ruined by bacterial spreaders. Various modifications of the technique failed to eliminate the bacterial spreaders.

The technique now used at the Ohio Agricultural Experiment Station involves a triple-agar-layer plate. Briefly, the 3 layers are: 1) a sterile-water agar layer, 2) a soil-suspension agar layer, and 3) an assay-fungus agar layer. This technique, used to assay for antagonists of *Fusarium roseum* f. *cerealis* (Cke.) Snyd. & Hans., *Rhizoctonia solani* Kuehn, *Pythium ultimum* Trow, and *Verticillium albo-atrum* Reinke & Berth., can undoubtedly be used for many other pathogenic fungi. Antagonism assays of 360–600 plates per assay have been routinely made with the triple-agar-layer plate method.

ISOLATION TECHNIQUES.—The agar layers were pumped into the plates with an automatic pipetting device consisting of a 5-ml B-D Sana-Lok Pitkin Syringe[3] (No. P5SL1) equipped with a B-D Filling Outfit Assembly[3] (No. 1220FO). The plunger opening of the Pitkin syringe was plugged with rubber for use in combination with this filling outfit. All water used for soil dilutions, agar layers, and fungus cultures was distilled and then de-mineralized, and had a resistance of 1.5–2.0 million ohms when collected. Water of this purity may not be required, but all toxic ions must be removed. Difco Bacto-Agar was used in all agar layers.

The first agar layer, 10 ml of 1.5% water agar, was pumped into the plates at least 1 day before the second layer was added. The first layer serves several functions: 1) it provides a level surface for succeeding layers, 2) it prevents growth of anaerobic bacteria at the bottom of the plate, and 3) it results in zones of antagonism that are generally more distinct.

The second layer, 5 ml of 1% water agar, contained the soil suspension. In field soil studies with *F. roseum*, *R. solani*, and *P. ultimum* the dilution employed was 1:20,000, which generally yielded average counts of 4–10 antagonists per plate from 10 plate sets, depending on the assay fungus used and the treatments applied to the soil. A 1:100,000 dilution was used in greenhouse soil studies with *V. albo-atrum*. For different pathogens, soils, and experimental conditions, the dilution factor must be determined experimentally. Too many antagonists per plate results in coalescent inhibition zones, making counting difficult; too few antagonists are difficult to handle statistically.

Soil dilutions were made in sterile de-mineralized water starting with a 1:10 dilution (either 10 g of soil in 100 ml water or 5 g of soil in 50 ml water). This 1:10 dilution was shaken 5 minutes on a mechanical shaker. Each subsequent dilution in water was shaken 2 minutes. The final dilution was in 1% water agar kept liquid in a water bath at 47°C. The flasks of agar were then thoroughly swirled by hand, and a set of 10 plates made from each. Since 5 ml of the soil suspension in agar was added to each plate, this was taken into account in calculating the dilution factor. The plates were then incubated 2 days at 25°C. At the end of this period actinomycetes are barely visible on the plates. The length of this first incubation period greatly affects the outcome of the assay: if it is shortened, fewer antagonists develop; if lengthened, soil fungi tend to develop and interfere

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[3] Becton, Dickson and Company, Rutherford, New Jersey.

with the assay. The size of the zones of antagonism also generally increased with lengthened incubation, leading to counting difficulties created by greater coalescence of the zones and the inclusion of non-antagonistic actinomycetes within them.

The third agar layer was added after the 2-day incubation of the soil-suspension agar layer. Three-day-old cultures of the assay fungus cultured at 25°C in Czapek's sucrose-nitrate solution were macerated in a Waring micro-blender and mixed with an equal volume of Czapek's agar (2% agar) kept liquid at 47°C. Five ml of the Czapek's-agar–macerated-fungus suspension were pumped into each plate. The plates were then incubated at 25°C—4 days for *F. roseum*, *R. solani*, and *P. ultimum*, and 7 days for *V. albo-atrum*. The numbers of antagonists were then counted. Representative plates are shown in Fig. 1.

The success or failure of the technique depends greatly on the manner of culturing and macerating the individual pathogens. Presumably, a major reason why culturing and maceration affect the assay is that these procedures affect the resultant number of viable mycelial fragments in the assay-fungus agar layer. Soil fungi and bacteria do not interfere with the assay when the assay fungal pathogen develops quickly and uniformly in this third layer. Enough viable mycelial

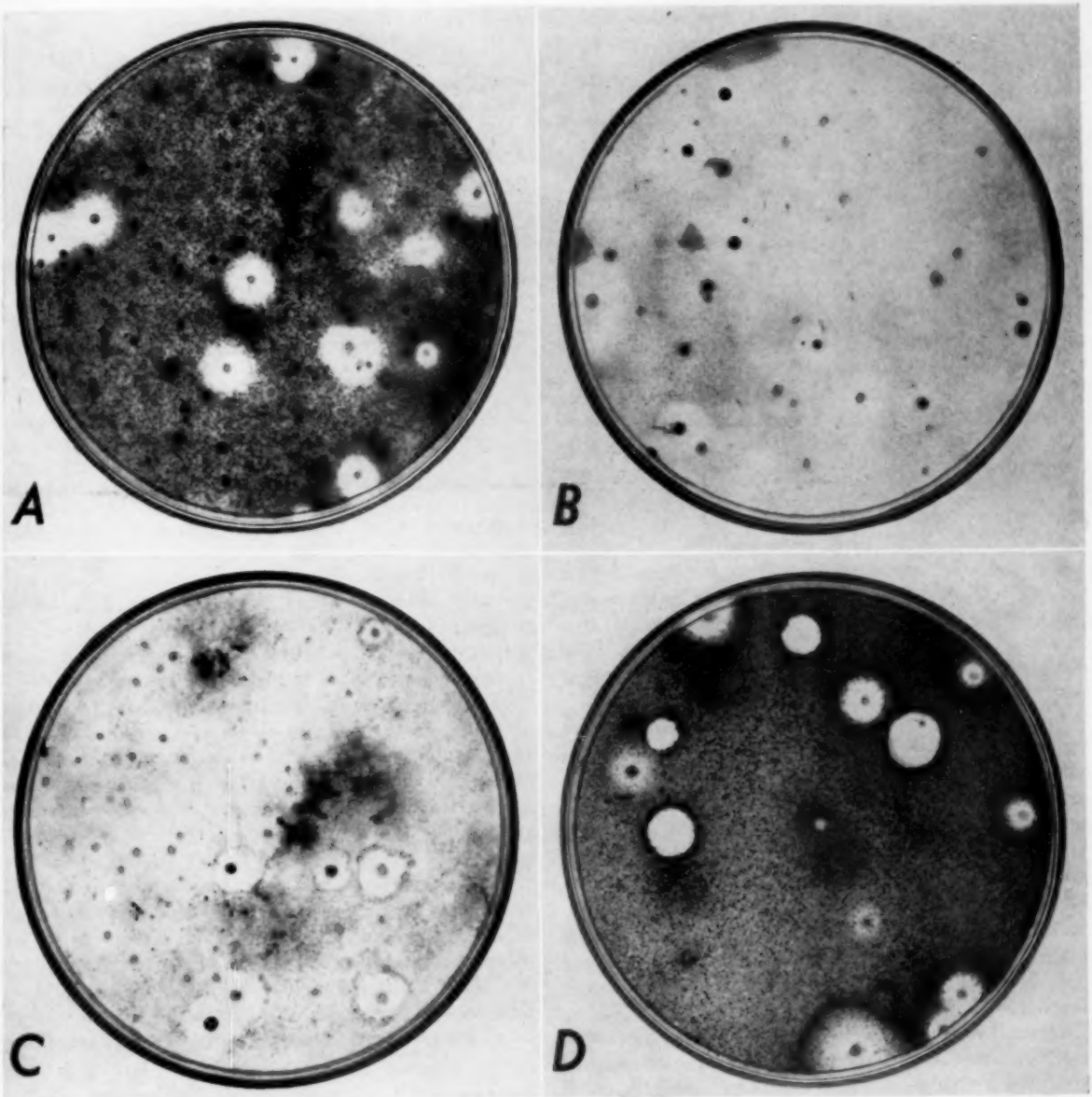


**Fig. 1.** Representative antagonism assay plates. **A)** *F. roseum* as assay pathogen; antagonists are visible at center of zones, non-antagonistic actinomycetes show as dark spots o ergrown by the fungus. **BCD)** Respectively, *P. ultimum*, *R. solani*, and *V. albo-atrum* as the assay pathogens.

fragments must be obtained for fast and uniform development of the assay fungus, but too many fragments may plug the pipetting device or result in obscure antagonism zones. For the pathogens used to date, the following culturing and maceration techniques are used.

1) *V. albo-atrum* and *F. roseum*—cultured 3 days at 25°C in 50 ml Czapek's solution in 250-ml Erlenmeyer flasks as still cultures. Macerated for 2 15-second periods.

2) *R. solani*—cultured 3 days at 25°C in 25 ml Czapek's solution in 250-ml Erlenmeyer flasks as still cultures. Macerated for 3 5-second periods and 1 10-second period.

3) *P. ultimum*—pre-cultured 1 day in 5 ml half-strength Czapek's solution in test tubes at 25°C, 2 inoculum pieces per tube. Transferred to 250-ml Erlenmeyer flasks containing 25 ml Czapek's solution and incubated 3 days at 25°C as shake cultures. Macerated for 1 10-second period.

Occasionally, a few colonies of bacteria antagonistic to the pathogenic assay fungi are isolated in addition to the antagonistic actinomycetes. It has been observed that bacterial antagonists are generally more common in *P. ultimum* assay plates than in assay plates of the other fungal pathogens.

The isolation techniques presented have been used successfully in repeated large-scale assays. Methods used successfully in small-scale assays based on a limited number of soil samples are not necessarily successful in larger-scale assays involving soil samples from more widely varied cultural and cropping treatments. Emphasis has consequently been placed on developing workable techniques for the 4 pathogens thus far used; possible refinements of the techniques have received little attention.

The use of this general technique for other fungal pathogens should be a matter of selecting culturing and maceration techniques yielding optimum numbers of viable mycelial fragments for quick and uniform development of the pathogens in the assay-fungus agar layer.

QUANTITATIVE AND STATISTICAL ASPECTS OF THE ASSAY METHOD.—Fully as important as the isolation techniques are the procedures employed to obtain quantitative data suitable for statistical analysis. Here must be considered such things as: soil-sampling technique, treatment replication, numbers of plates per replicate, number of antagonists per plate, and type of statistical distribution of antagonists.

The soil-sampling technique used is based on the pooled soil-sampling technique that Schmitthenner and Williams (5) developed for studies of soil fungi. For field-plot studies, 16 vertical soil cores, 1 in. in diameter by 3 in. in length, were taken from each 14×50-ft. plot and pooled into 1 composite sample, from which a dilution was then made. Smaller pooled samples were employed for greenhouse studies.

Treatment replication is subject to many variables, with each experiment presenting its own problems.

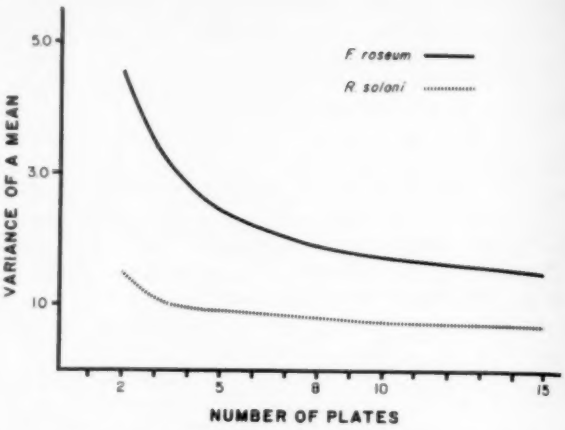


**Fig. 2.** Effect of number of plates on the variance of a mean for *F. roseum* and *R. solani*.

From results to date it is suggested that each treatment be replicated at least 6 times. This of course depends on the magnitude of the differences between treatments and the magnitude of the difference one wishes to detect. The cost factor must also be considered, particularly in field studies.

The number of plates to use per soil sample was investigated in a field-plot experiment with 5 crops replicated 4 times, using 10 assay plates per plot for each of 2 fungi, *F. roseum* and *R. solani*. The components of variance due to plates and experimental error were ascertained. The variance of a plot mean can be shown to be: $\frac{\sigma_p^2}{p} + \sigma^2$, where $\sigma_p^2$ is the component of variation due to plates, $\sigma^2$ is the component of variation due to experimental error, and p is the number of plates. Estimates of $\sigma_p^2$ and $\sigma^2$ were found to be $s_p^2 = 7.14$, $s^2 = 1.01$ for *F. roseum*; $s_p^2 = 1.85$, $s^2 = 0.573$ for *R. solani*. Using these components to estimate the variance of a mean with various numbers of plates, the estimates given in Fig. 2 were obtained. Beginning with small numbers of plates, the variance of a mean decreases rapidly as plate numbers increase, then decreases more slowly, and finally reaches a point where it is uneconomical to increase plate numbers further. In this experiment, the point at which little gain was realized by increased plate numbers was reached sooner with *R. solani* as the assay fungus than with *F. roseum*. It was deemed inadvisable to employ different numbers of plates for different assay fungi; further, allowance was made for the probability that the variance of a mean might differ if determined from other experiments. Based on these estimates of the variance of a mean, 10 was chosen as a satisfactory number of plates per soil sample.

The number of antagonists per plate has been discussed in part under isolation techniques. A low (2–3) average number of antagonists per plate from the control soil does not provide an adequate basis for comparing treatments. The difficulties arising

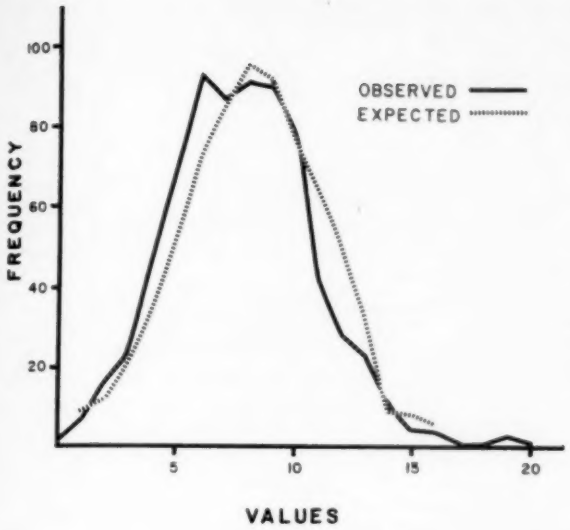


**Fig. 3.** Distribution of observed numbers of antagonists compared to the calcuated normal distribution of expected numbers.

from too high an average number of antagonists have already been discussed. An average number of 8–10 antagonists per plate for the control soil has yielded good results.

The distribution of counts of antagonists was studied using data from a greenhouse experiment consisting of 72 uniformly treated cans of soil, 10 plates per can, with *V. albo-atrum* as the assay fungus. The statistics from the distribution were: mean, 7.76; variance, 9.27; $g_1$, 1.34; $g_2$, 0.98; standard deviation, 3.04. The significance of $g_1$ and $g_2$ as measure of skewness and kurtosis proved highly significant in t tests, indicating that the distribution is skewed to the right and is peaked. The mean and standard deviation were used to estimate a true mean and standard deviation. From these estimates, numbers expected under a normal distribution were calculated. The observed and expected distributions are plotted in Fig. 3. The analysis of variance assumes a variable that is normally distributed. The data do not fulfill that assumption. Box and Anderson (1) devised a statistic for evaluating the effect of non-normality on the analysis of variance. This statistic, $\delta$, was computed for the above case and found to be 1.0014, which leads to the conclusion that non-normality of distribution would have little effect on analysis of variance of data of this nature.

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# EFFECT OF INOCULUM LEVEL ON THE SEVERITY OF TOBACCO BLACK SHANK [1]

G. V. Gooding and G. B. Lucas

SUMMARY

Tobacco varieties representing various levels of black-shank resistance were inoculated with different concentrations of zoospores of *Phytophthora parasitic* var. *nicotianae* in greenhouse tests. Rapidity of disease development was directly related to number of spores used. It required more spores to kill resistant plants than susceptible plants in a given time. Environmental factors that affected the succulence of the plants also affected the disease indices.

One of the most difficult tasks in developing tobacco varieties resistant to blackshank, incited by *Phytophthora parasitica* (Dastur) var. *nicotianae* (Breda de Haan) Tucker, is evaluation of disease resistance. The type of resistance in currently used tobacco varieties is apparently inherited quantitatively (12). This imposes 2 important requirements on the disease testing program: susceptible individuals must be eliminated from segregating populations, and varying levels of resistance must be measured accurately. The disease conditions provided must therefore be critical.

Most testing for black-shank resistance has been done in naturally infested field plots. Because disease intensity varies widely from location to location and season to season, reliable data require many test locations and large plant populations. Variations have resulted primarily from lack of control over inoculum potential and environment. Various means of artificial field inoculations have been devised to overcome the former problem; however, lack of environmental control greatly limits success (1).

A valuable adjunct to field testing would be a standardized greenhouse technique for evaluating disease resistance. No concerted effort seems to have been made to develop such a technique, though greenhouse inoculations have been used by different investigators (2, 3, 7, 8, 9, 10, 11), e.g., in greenhouse tests, Valleau (11) found *Nicotiana longiflora* immune to *P. parasitica* var. *nicotianae*, whereas Lautz (8) found *N. longiflora* highly resistant but not immune. Lautz suggested that temperature might be responsible for the differences obtained. Other possible explanations include differences in virulence of the isolates (2), in soil pH (6), and in the physiological condition of the host plants. These factors have been shown experimentally to affect disease development. Another possibility undoubtedly is the inoculum potential to which the plants were exposed. Knowledge of the effect of inoculum potential on infection and subsequent disease development would be another step toward development of a standardized technique for evaluating resistance to black shank.

This paper reports the effect of different levels of inoculum on black shank development in susceptible and resistant tobacco varieties in greenhouse tests. It also reports the reaction to a given amount of inoculum of 5 flue-cured varieties of known field resistance.

MATERIALS AND METHODS.—Seedlings grown in steam-sterilized soil were transplanted when about 2 in. high to 7-oz. paper cups containing sterilized sand. Each cup, with a hole cut in the bottom, was placed in a 3½-in. clay saucer. The plants were supplied on alternate days with nutrient solution (Hoagland and Arnon's (5) experimental nutrient solution No. 1) and tap water. At inoculation time, plants were about 4 in. tall.

Aqueous suspensions containing $10^5$, $10^4$, $10^3$, and $10^2$ zoospores per ml were prepared according to the method of Gooding and Lucas (4). A 5-ml continuous pipette fitted with a 14-gauge 4-in. cannula was used to inject 10 ml of inoculum into the sand in the vicinity of the tap root. After 3 weeks, all plants surviving were transplanted to soil in 4-in. clay pots and disease readings continued weekly for 3 weeks. The roots of the surviving plants were then washed, and the amount of root infection recorded.

Used in the first tests were 2 flue-cured tobacco varieties: Dixie Bright 101, a moderately resistant variety, and 402, a susceptible variety. Used in later tests were 5 flue-cured varieties, ranging from susceptible to highly resistant: 402, Bottoms Special, Vesta 5, Coker 139, Dixie Bright 101, and Dixie Bright 102. When 5 varieties were tested, each plant was inocu-

TABLE 1.—*Disease indices obtained by inoculating 2 varieties of tobacco with different amounts of zoospores of P. parasitica var. nicotianae*

| Variety | Experiment | Disease index[a] by inoculum level[b] | | | |
|---|---|---|---|---|---|
| | | $10^2$ | $10^3$ | $10^5$ | $10^6$ |
| 402 | 1 | 1.0 | 13.0 | 82.5 | 89.3 |
| | 2 | 17.3 | 45.0 | 87.5 | 92.7 |
| | 3 | 3.8 | 22.5 | 87.0 | 90.0 |
| | Mean | 7.5 | 31.5 | 86.0 | 90.6 |
| Dixie Bright 101 | 1 | 0.0 | 0.0 | 2.0 | 6.3 |
| | 2 | 0.0 | 0.3 | 0.5 | 20.3 |
| | 3 | 0.0 | 0.0 | 1.5 | 8.3 |
| | Mean | 0.0 | 0.1 | 1.4 | 11.6 |

[a] 0 = no plants dead 20 days after inoculation; 100 = all plants dead 1 day after inoculation.
[b] Number of zoospores per plant.

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TABLE 2.—*Disease indices[a] obtained for 5 tobacco varieties 20 days after inoculation with $10^6$ zoospores of the black shank fungus per plant*

| Varieties | Tests | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| 402 | 87.5 | 89.0 | 87.0 |
| Bottoms Special | 76.0 | 76.5 | 68.0 |
| Vesta 5 | 69.5 | 65.8 | 34.0 |
| Dixie Bright 101 | 40.3 | 41.5 | 24.0 |
| Coker 139 | 40.7 | 39.5 | 20.0 |
| Dixie Bright 102 | 18.0 | 30.0 | 12.5 |

[a] 0 = no plants dead 20 days after inoculation; 100 = all plants dead 1 day after inoculation.

lated with $10^6$ zoospores, and disease indices were calculated 20 days after inoculation.

A highly pathogenic isolate of the flue-cured strain, obtained from Dr. J. L. Apple, was used throughout the inoculation studies.

Disease readings were taken daily for 20 days following inoculation. A plant was considered dead when all but the bud leaves had permanently wilted. A disease index was computed to reflect the potential of a given level of inoculum. Classification was by the number of days from inoculation to plant death (1 to 20); the numerical values corresponding to the 20 classes were, respectively, from 100 down to 5, at intervals of 5. Thus, classes 1, 2, 3, 4, etc., have respective values of 100, 95, 90, 85, etc.

In calculating the disease index for a given inoculum level, the 20 plants inoculated at each inoculum level are allocated among the proper classes, the numbers in each class multiplied by the corresponding numerical value, totaled, and divided by 20. By this system, if all 20 plants at a given level are killed in 1 day, the disease index is 100 ($20 \times 100 \div 20$). This system is similar to that used by Apple (2).

Experiments were conducted in the greenhouse during the summer of 1957. Daytime temperatures during the experiments were as high as 110°F; therefore, all inoculations were made at night, after the temperature had fallen below 85°F. The tests were run 3 times. Each test consisted of 20 plants of each variety at each inoculum level. A split-plot design was used, with varieties as whole plots and levels of inoculum as sub-units. Four replicates were used, consisting of 5 plants of each variety at each level.

RESULTS.—There was a direct relation between rapidity of disease development and inoculum level. Although the disease index for each variety at a given level did not remain constant from experiment to experiment, its relative position remained the same within experiments (Table 1).

Rapidity of disease development was important in separating disease development at different inoculum levels. Using $10^4$ spores per plant, about 50% of the plants of susceptible variety 402 were killed in 3 weeks; when $10^5$ spores per plant were used, every plant was killed in less than a week. When the total number of plants killed at 20 days was used as the only criterion of disease development, there was no difference between the inoculum potential of $10^5$ and $10^6$ spores on Variety 402 (Fig. 1); however, the disease index based on the rapidity of disease development revealed a difference. With Dixie Bright 101, $10^6$ zoospores per plant were required to kill or cause severe disease in 100% of the plants after 6 weeks; variety 402, however, required only $10^5$ spores for the same results. As shown in Table 2, in 2 tests out of 3, disease indices were almost identical for the 5 different test varieties. In the third test, the more resistant varieties showed lower disease indices, but, here again, their relative positions remained the same within the experiment.

DISCUSSION.—These studies indicate that the number of zoospores used to inoculate a tobacco plant affects directly the rapidity and severity of disease development. It should be emphasized that the results were obtained under one set of environmental conditions only. Alteration of these conditions can greatly affect disease development. As an example, in preliminary tests, the succulence of the test plants greatly

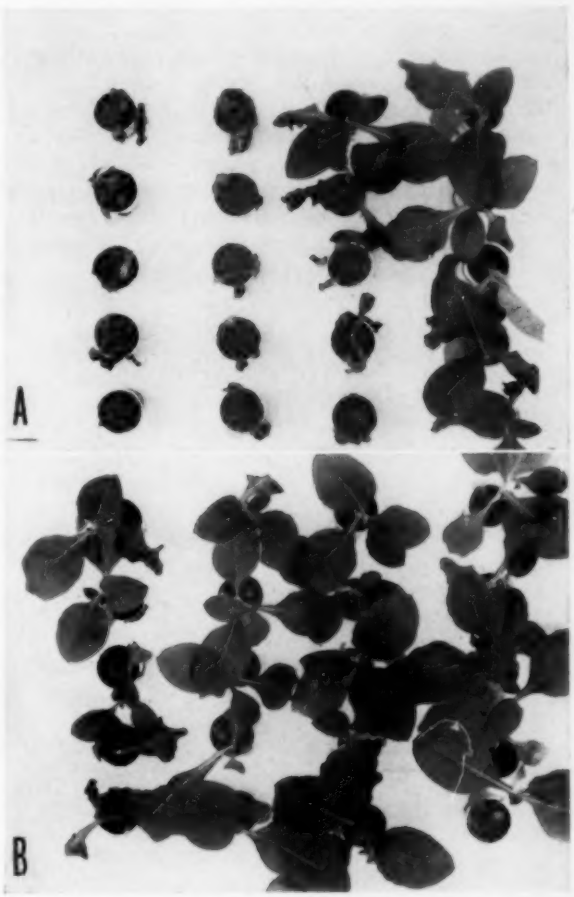


**Fig 1.** Disease development 2 weeks after plants of 2 tobacco varieties were inoculated with different concentrations of zoospores of the black shank fungus. Vertical rows left to right, $10^6$, $10^5$, $10^4$, and $10^3$ zoospores per plant. **A)** Variety 402, **B)** Variety Dixie Bright 101.

influenced disease development. In one experiment, half of the test plants, Dixie Bright 101, were supplied with nutrient solution each day and the other half supplied on alternate days with nutrient solution and plain water. Two weeks after inoculation with a zoospore suspension consisting of $10^5$ spores per ml, all the plants receiving nutrient solution daily were dead, whereas only 20% of the other plants were killed.

Although the disease indices held their relative positions in inoculum level and varietal studies, there was considerable variation in indices between experiments. Much of this variation probably resulted from differences in the environment before and after inoculation. Light intensity (extended cloudy periods) and temperature were probably the most important variables involved.

It seems that, with the knowledge available on factors affecting black shank, the main requirement now necessary for a standardized means of evaluating disease resistance is control over the environment. The procedure used has the following advantages: 1) growing plants in sand watered with nutrient solution affords a means of providing uniform plants by eliminating effects of various soil types, 2) zoospore suspensions of a given concentration are easily obtainable, 3) zoospores infect the host in a matter of hours, and therefore are exposed to environmental conditions for a relatively short period, and 4) a disease index based on the rapidity of disease development provides a more accurate means of distinguishing between varieties with similar levels of resistance.

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# FACTORS INFLUENCING SPORANGIAL FORMATION AND ZOOSPORE ACTIVITY IN PHYTOPHTHORA PARASITICA VAR. NICOTIANAE [1]

G. V. Gooding and G. B. Lucas [2]

SUMMARY

Sporangia of *Phytophthora parasitica* var. *nicotianae* were produced in abundance when aerial mycelium of 6–20-day-old cultures was stripped from oatmeal agar plates, wetted, and incubated 6–10 days at 24–26°C, provided an isolate was used that carried the potential for abundant sporulation. Air appeared necessary for sporangial production, and light did not. In 7 solutions tested, sporulation was maximum in .01*M* $KNO_3$ solution, and minimum in double-distilled water. The cardinal temperatures for motility and germination of zoospores were about 8, 20, and 34°C; however, more than 90% germinated between 16 and 30°C. Zoospores remained motile longer and more germinated in 1% dextrose solution than in distilled water, tap water, or complete nutrient solution. At $10^6$ spores per ml distilled water, more than 50% of the spores were motile after 4 hours; at $10^5$, no zoospores were motile after 5 minutes. Germination was about 98% at $10^5$ or more zoospores per ml, and below 50% at lower concentrations.

INTRODUCTION.—De Bary (4) first described zoospore production in the genus *Phytophthora* in 1860. Since then, many workers have investigated the effects of various factors on sporangial formation. As reviewed by Wilson (15) and others (2, 11, 13, 14), the principal factors involved are substrate, moisture, air, light, temperature, and age of sporangia. However, as shown by Leonian (9), the sporulation response to these interdependent variables may vary widely among even closely related members of the genus. Leonian concluded that "the chief limiting factor in the reproduction of *Phytophthora* is access to atmospheric oxygen." Fawcett and Klotz (5) induced zoospore formation in several *Phytophthora* spp. by warming and then chilling them, a phenomenon first noted by Melhus (12), in 1911. Wills (14) made an extensive study of the influence of environmental factors on sporangial formation in *Phytophthora parasitica* (Dastur) var. *nicotianae* (Breda de Haan.) Tucker. He found that the cardinal temperatures were about 15, 20–25; and 30°C; that sporulation occurred under all conditions of light and darkness; and that the number of sporangia varied at different pH values, though no optimum pH was evident. Of several solutions tested, a soil extract and an ion-balanced salt solution supported abundant sporulation. Tap water supported good sporulation, as did tap water concentrated 10 times; whereas distilled water and double-distilled water supported meager to no sporulation. Wills suggested that sporangial formation probably depends in part on the proper balance of ions, and that carbonates may trigger the biochemical transformations leading to sporulation.

Little work seems to have been done on the effect of environmental factors on zoospore activity in *P. parasitica* var. *nicotianae*. However, Crosier (3) found that, with *P. infestans* (Mont.) d By., zoospores were very sensitive to temperature, and Goode (6) noted that, with *P. fragariae* Hickman, mechanical disturbance, such as stirring or shaking, induced more rapid zoospore incystment.

A requisite for a study on inoculum potential with *P. parasitica* var. *nicotianae* (7) was a technique for consistently obtaining active zoospores in quantity. Techniques of previous workers were not satisfactory. Therefore, this investigation was undertaken to deter-

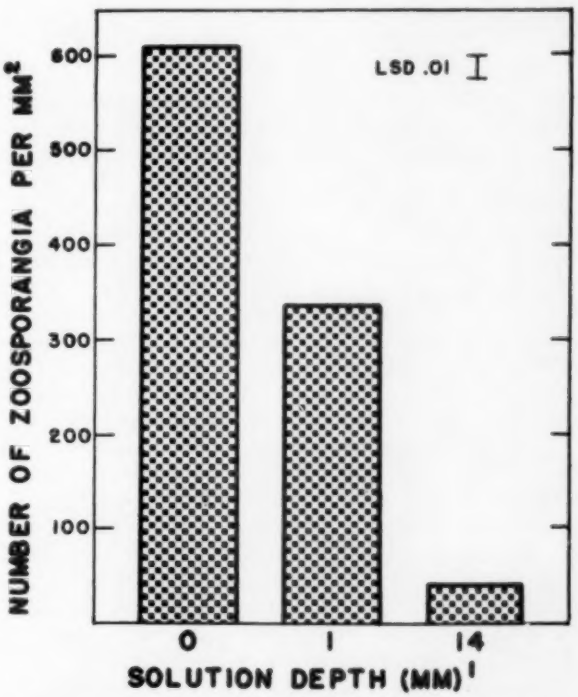


**Fig. 1.** Effect of solution depth on zoosporangial formation by *Phytophthora parasitica* var. *nicotianae*. Eight-day-old mycelial mat incubated in .01*M* $KNO_3$ solution, placed in a Petri dish, and held in continuous light for 10 days at 26°C; 0—mat wetted, 1—about ⅓ of mat protruding above solution level, 14—top of mat 14 mm below surface of solution.

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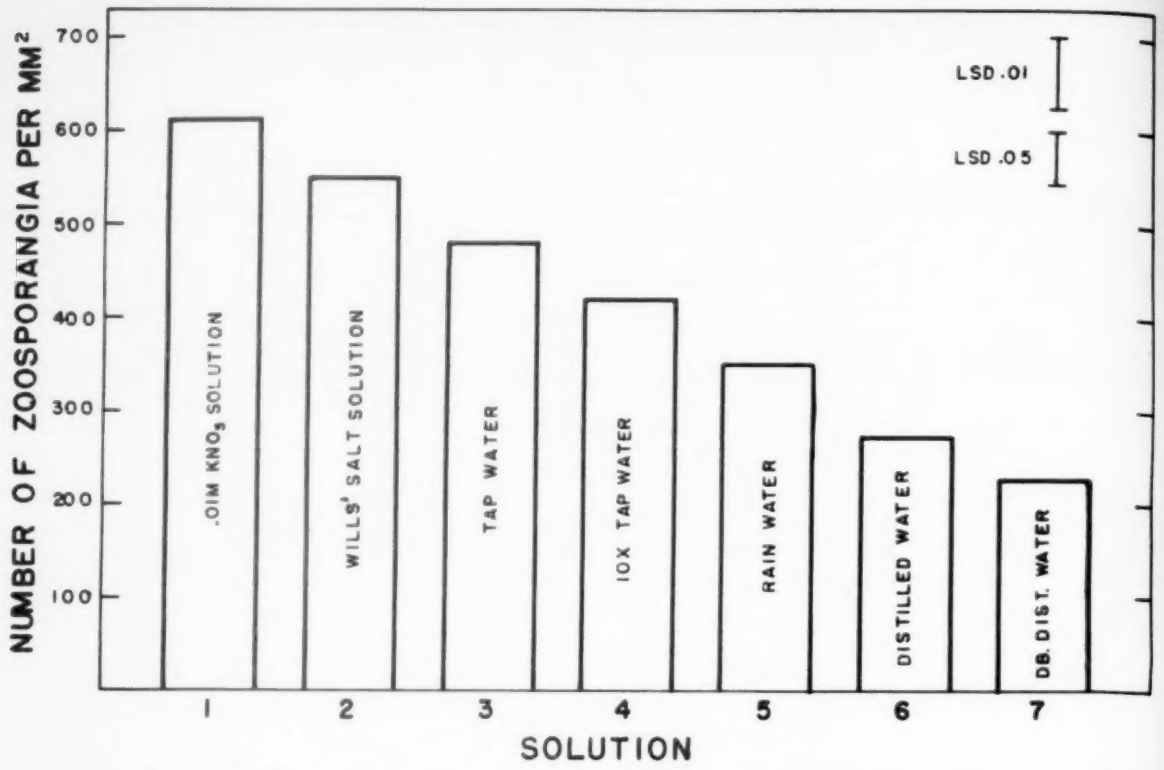


**Fig. 2.** Effect of different incubation solutions on zoosporangial formation by *Phytophthora parasitica* var. *nicotianae*. The 8-day-old micelial mats were wetted, placed in Petri dishes, and incubated in continuous light for 10 days at 26°C.

mine the effect of certain environmental factors on sporangial formation and zoospore activity, and to develop a technique for mass zoospore production.

MATERIALS AND METHODS.—Oatmeal agar was selected as a medium since it supports rapid fertile mycelial growth of the fungus. Seventy-five g of rolled oats in 600 ml of water were ground 5 minutes with a Waring blender. The resulting blend was heated to 45–55°C and added to 20 g of agar previously melted in 400 ml of distilled water. About 300-ml aliquots of the medium were autoclaved 90 minutes at 121°C in 16-oz. pharmaceutical bottles with caps *screwed on tightly* to prevent frothing.

After the fungus had grown in Petri dishes for 10 days at 26°C, the aerial mycelium, unless otherwise specified, was stripped from the culture and transferred to a Petri dish containing distilled water or test solution, and incubated at least 48 hours at room temperature (10). Circular mats of mycelium, for sporangial assay, were obtained by cutting through the aerial mycelium with a cork borer and stripping off the mycelium with a dissecting needle.

*Sporangia.*—The following factors on sporangial formation were tested:

1) *Incubation solutions and their depth.*—a) double distilled water, b) distilled water, c) tap water, d) tap water concentrated by evaporation to 1/10 volume, e) rain water collected in chemically cleaned beakers, f) .01*M* $KNO_3$ in distilled water, and g) Wills' (14) salt solution. The mycelial mats were supplied 3 different amounts of each solution: a) enough to wet the mycelial mats, b) about $\frac{2}{3}$ of the mat was submerged, and c) the mats were covered to a depth of 14 mm. In the last, the mats were prevented from floating by placing glass cylinders on them.

2) *Age of mycelium.*—The effect of culture age was tested by cutting mats from the same culture plates at equal distances from the inoculum plug at 6, 8, 10, 15, and 20 days after inoculation.

3) *Duration of incubation.*—Effect of incubation period was determined by making readings of sporangial incidence 2, 4, 6, 8, 10, and 12 days after wetting the mats.

4) *pH.*—The response to pH was tested over a pH range of 4.0–10.0, at 1.0-pH intervals. Equal volumes of .01*M* monobasic and .01*M* dibasic potasium phosphate solutions were mixed to serve as a buffer. The pH range was established with 0.1*N* HCL or 0.1*N* NaOH. Five ml of solution at each pH level were placed on the mats. Before placing the mycelial mat at a given pH, the mats were rinsed in 50 ml of the solution having this pH.

5) *Isolates.*—Five single-zoospore isolates of the fungus were compared for relative sporulation. Cultures of the isolates, furnished by Dr. J. L. Apple, consisted of a burley strain from Florida, and 4 isolates of the flue-cured strain from North Carolina (1).

6) *Temperature.*—Sporangial formation was studied

at temperatures of 8–36°C, at 4° intervals. Test plates were placed in sealed polyethylene bags to prevent drying of the mats.

7) *Light.*—Continuous light (about 250 ft-c) versus continuous dark was tested at 26°C.

Unless otherwise specified, the following were the same for all tests: 1) flue-cured isolate 601; 2) the mycelial mats were placed in a dish containing 10 ml of sterile distilled water, and positioned, and then all water not absorbed by the mats was decanted; and 3) zoosporangial readings were taken 5 days after the stripped mycelial mats were exposed to the test conditions. Each treatment was replicated 6 times, and each experiment repeated 3 times. Zoosporangial counts were made with the aid of a grid reticule mounted in a 12× ocular of a stereoscopic microscope at 96× magnification. The counts were taken from the upper surface of the mycelial mat. The assay Petri plates were cleaned in potassium-dichromate–sulfuric-acid cleaning solution and rinsed in tap water, with final rinses in distilled water or double-distilled water.

*Zoospores.*—Temperature, various solutions, and spore dilution were studied as factors influencing zoospore motility and germination. Zoospores were obtained from mycelial mats bearing abundant sporangia by adding 10 ml of distilled water to the mat in a Petri dish and chilling 25 minutes at 8°C. About 15 minutes after the plates were returned to room temperature (about 26°C), most of the sporangia had discharged zoospores. The zoospore suspension was decanted into a beaker and the concentration determined; it usually contained more than $2 \times 10^6$ zoospores per ml. Zoospore counts were made with a Spencer Bright-Line Haemacytometer; the spores were immobilized by concentrating light from the source lamp (200-watt) onto the counting stage. Germination and motility counts were made with a stereoscopic microscope at 96× magnification.

Temperature studies were made over the range of 8–36°C at 4° intervals. Five minutes after the zoospores (about $10^5$ per ml) were subjected to a given temperature, an estimate was made of the per cent of spores that were still motile. Additional readings were made at the end of 1, 2, 4, 8 and 12 hours. Germination counts were made about 12 hours after all the spores at a given temperature had come to rest.

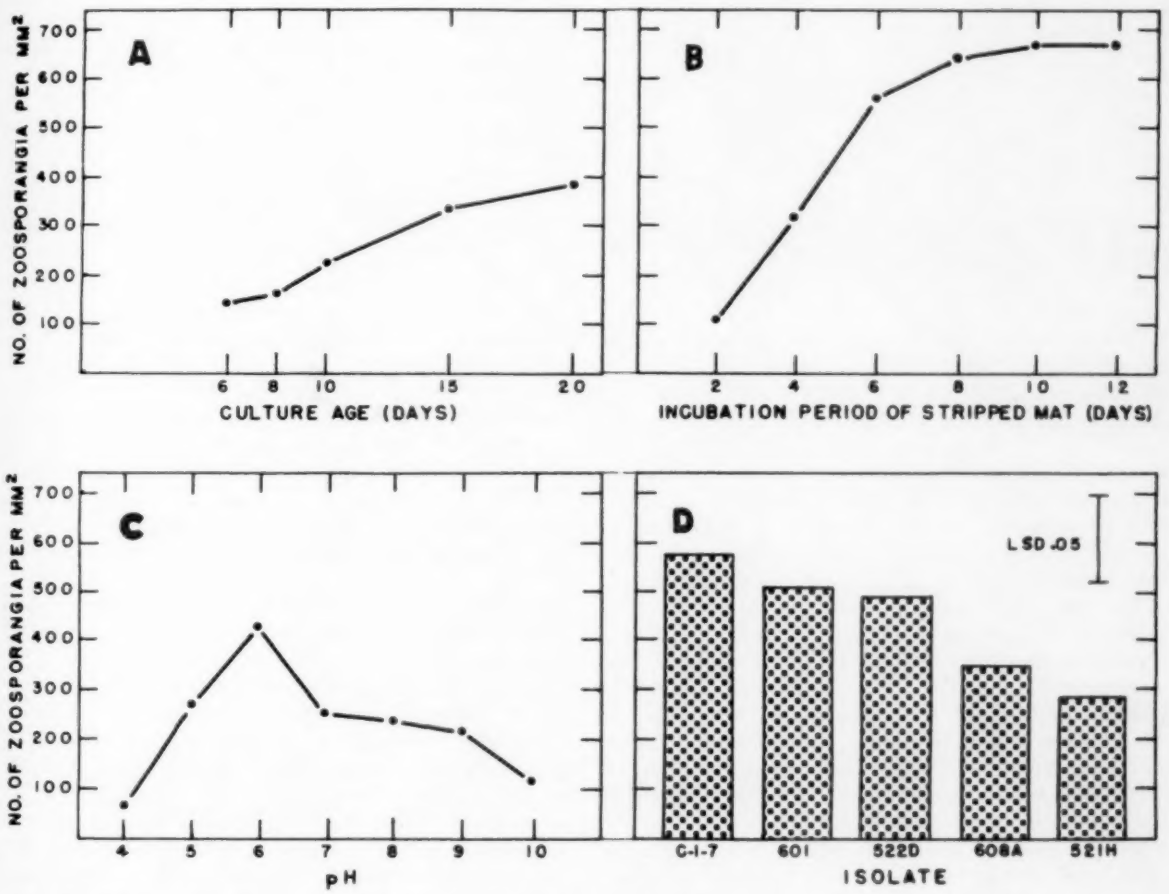


**Fig. 3.** Effect of **A)** culture age, **B)** incubation period, **C)** pH, and **D)** isolate on zoosporangial formation by *Phytophthora parasitica* var. *nicotianae*. The mats were wetted, placed in dry Petri dishes, and incubated in continuous light at 26°C.

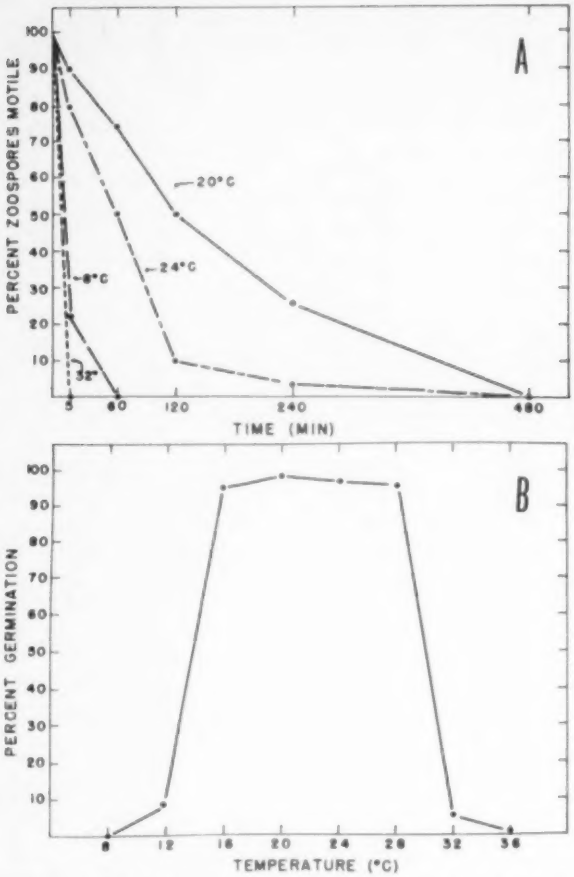


**Fig. 4.** Effect of temperature on motility **(A)** and germination **(B)** of zoospores of *Phytophthora parasitica* var. *nicotianae*. The zoospores were suspended in distilled water.

A study was made of the effect on motility and germination of the following solutions at 24°C: 1) distilled water, 2) tap water, 3) Hoagland and Arnon's (8) nutrient solution made with tap water, and 4) a 1% dextrose solution made with distilled water. Experimental data were recorded as in the temperature studies. The effect of zoospore concentration on activity was determined by serially diluting a stock zoospore solution 4 times, which gave concentrations ranging from $10^6$ to $10^2$ spores per ml.

Results.—The depth of the incubation solution markedly influenced sporangial formation (Fig. 1). The most sporangia formed on mycelial mats that were wetted only. Few, if any, sporangia formed on mats submerged 14 mm. The same trend held for all solutions tested. The liquid in which the mycelial mat was incubated also influenced sporangial development. The most sporangia formed in .01*M* $KNO_3$ solution, and the least in double-distilled water (Fig. 2), when the mycelial mats were only wetted. The same relation occurred at the intermediate depth of liquid; however, no differences were discernible between solutions when the mats were 14 mm below the liquid surface.

Age of mycelium also influenced sporangial production. More sporangia formed as mycelial age increased from 6 to 20 days (Fig. 3-A). It was noted that mycelium from 40-day-old cultures formed sporangia. Sporangial development by the mycelial mats increased as incubation time increased up to 10 days (Fig. 3-B), with the greatest rate of sporangial formation occurring up to 6 days. Optimum hydrogen ion concentration was at pH 6 (Fig. 3-C). Sporulation was good from pH 5 to pH 9, with least sporangia at the two extremes, pH 4 and pH 10. Isolates of strains of the fungus differed in their capacity to form sporangia (Fig. 3-D). Light did not influence sporangial formation, and the cardinal temperatures were found to be about 15, 20–25, and 32°C, confirming Wills' (14) results.

Zoospores retained motility longest at about 20°C, and more than 90% germinated between 16 and 30°C (Fig. 4). Zoospores remained motile longer, and more germinated in the 1% dextrose solution than in any of the other solutions. In tap water, there were no motile spores after 5 minutes, and no germination had occurred at 24 hours. Results in freshly prepared nutrient solution were similar to those in tap water; however, in old nutrient solution, results were similar to those in 1% dextrose solution.

A direct correlation was found between zoospore concentration and motility. At a concentration of $10^6$ spores per ml, more than 50% of the spores were motile after 4 hours. When this was diluted to $10^3$, no zoospores were motile after 5 minutes. The results were similar when .01*M* dextrose was substituted for distilled water as the suspending medium. Germination was about 98% at spore concentrations of $10^6$ and $10^5$, and below 50% at lesser concentrations.

Discussion.—Mycelial mats of *P. parasitica* var. *nicotianae* will form sporangia consistently and abundantly if 15–20-day-old aerial mycelium is stripped from oatmeal agar plates, wetted with .01*M* $KNO_3$ solution, and incubated 6–10 days at 24–26°C, provided an isolate is used that carries the potential for abundant sporulation. In this investigation, the most important factor in inducing the black shank fungus to form sporangia was some component of the atmosphere. That this component is oxygen was suggested by observations similar to those made by Leonian (9), who observed that, as hyphae from mycelium suspended under a column of solution approached the surface of the liquid, they produced sporangia.

These experiments confirm those of Wills (14) in that solutions of different ionic composition supported sporangial formation to different degrees. However, they differ in that sporulation was good in distilled water, whereas Wills observed no sporangia formed in distilled water. Moreover, the $KNO_3$ solution supported as much sporulation as Wills' ion-balanced salt solution. The divergence of results may have resulted from differences in isolates or in the nutritional status of the fungus at the time of removal from its food source. Oatmeal appears to be a more favorable

medium for growth than the media Wills used.

In the pH experiment, pH 6 was optimum for sporangial formation. This relation did not hold in the solution test, however, since sporulation was good in distilled water with an initial pH of 6.8, and abundant in Wills' salt solution with an initial pH of about 8. The ionic composition of the solution may mask the pH effect, or be more important.

Zoospores of *P. parasitica* var. *nicotianae* are very sensitive to environmental conditions; however, under favorable conditions, some remain motile for 7 hours or more. The reasons for the response of zoospores to the imposed conditions was not determined. The effect of dilution on zoospore motility is probably related to some metabolite excreted by the mycelium, by the zoosporangia at time of discharge, or by the zoospores themselves. The long period of motility in the 1% dextrose solution may be related to osmotic pressure or nutrition. The consistently adverse effect of tap water (chlorinated) on zoospores and the results obtained with freshly prepared versus old nutrient solution indicate that chlorine might be the factor controlling this response. Since zoospores are the primary infective stage of the fungus, their sensitivity to chemicals might be utilized in the control of black shank.

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# DETECTION, PREVALENCE, AND SIGNIFICANCE OF LATENT VIRUSES IN PELARGONIUM [1]

A. Kivilaan and R. P. Scheffer [2]

SUMMARY

By reactions of seedlings grafted with scions from suspect plants, latent conditions, presumably of virus origin, were detected in 62% of 600 plants of common pelargonium varieties from commercial sources. Seedling reactions included ringspot, necrotic ringspot, crinkle, mottle, mosaic, curly top, stem necrosis, and top necrosis. Reactions were more clear-cut when scions were from lower parts of infected plants. Sap and dodder transmission failed. Most plants assayed infected later developed virus-like symptoms, which also showed under low-temperature–low-light conditions. Some viruses decreased plant tolerance to bacterial stem rot.

*Pelargonium hortorum* Bailey, being largely vegetatively propagated, is vulnerable to systemic diseases. Bacterial blight or stem rot is the limiting factor in production, and virus diseases are common. Studies on bacterial blight (4) and an abstract on work with viruses (3) have been published. This paper reports on further work with graft-transmissible disorders, presumably of virus origin.

Many brief reports in the literature describe various virus diseases of pelargonium, but little work has gone beyond description. This is understandable, because of the difficulties experienced in transmitting the viruses. Several pelargonium virus diseases have been known for many years; leaf curl and mosaic were described in detail by Jones (2), and beet curly top virus was found in geraniums by Severin and Freitag (1, 6). McWhorter (5) recently expressed the opinion that tomato spotted wilt and cucumber mosaic viruses are common in pelargonium. Still other viruses may affect pelargoniums, as indicated by the successful transmission to pelargonium of Southern celery virus (9) and of tobacco necrosis virus from roots of symptomless plants (7). Any particular symptom type may be the result of multiple infections, or several individual viruses may give similar symptoms.

The better known pelargonium viruses cause definite symptoms only during certain seasons (2, 5). Apparently this is also true of tobacco necrosis virus, which Szirmai (8) reported as the cause of light necrotic lesions on basal leaves under low temperature and low light. Basic to further study of these diseases are methods of detecting latent infections and of transmitting the common pelargonium viruses to other hosts. Described herein are a method of detecting symptomless infections, and efforts to transmit the viruses to other hosts.

MATERIALS AND METHODS.—Virus diseases were transmitted by grafting to virus-free seedling plants. Scions (2–3 in. long) were taken from plants under question, inserted by veneer grafts into stems of 4–6-in. seedlings, and bound with latex bandage. Two scions were taken from each plant and grafted into 2 seedlings, and each assay was repeated 2–3 times. Grafted seedlings were kept 2 months or more.

Stock cultures of *Xanthomonas pelargonii* (Brown) Starr & Burk. were kept at 4–6°C on autoclaved potato plugs from which cultures for inoculum were started in potato-dextrose broth. Isolates were also kept in stock plants, and pathogenicity was checked at intervals. Plants were inoculated by puncturing the stem with a needle and inserting a small cotton plug dipped in bacterial suspension. These methods have been described in more detail (4).

RESULTS.—*Detection of latent viruses.*—To detect any viruses in plants of standard varieties, veneer grafts were made to *P. hortorum* seedlings that were presumed to be virus-free. Many such transfers showed that unusual symptoms often appeared as initial reactions on new leaves of the seedling stock, both above and below the graft. Severe stem necrosis and/or top necrosis were sometimes associated with leaf symptoms. Such reactions indicate that latent or symptomless viruses were present in the scions. It was not possible to classify the symptoms by Jones' terminology (2), as leaf curl, mosaic, or curly top, nor was it possible to fit all symptoms into McWhorter's classification (5). Therefore, the initial leaf symptoms in seedling plants were classified as ringspot (Fig. 1-b), necrotic ringspot (Fig. 1-a), crinkle (after McWhorter), mottle (a tiny yellow fleck surrounded by light green area) (Fig. 1-c), mosaic (after Jones), and leaf cupping (Fig. 2) (after McWhorter). Except for severe stem and top necrosis, symptoms usually disappeared with new growth of infected seedlings. Stem and top necrosis, which sometimes killed seedlings, was associated only with leaf curl and necrotic ringspot, although these were often seen without stem or top necrosis. That such necrosis was caused by viruses was further indicated by many unsuccessful attempts to isolate bacteria or fungi.

The possibility of detecting latent infections by manipulation of light and temperature was examined in a limited way. Thirty-five known virus-infected and 35 virus-free plants (vars. Ricard, Radio Red, Olympic Red, Irvington Beauty, and Fiat) were kept 10 days under continuous low-intensity light (400 ft-c) at 9°C. Duplicate groups were kept in the greenhouse

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at 21°C night temperature. With many virus-infected plants, older leaves yellowed in low-light–low-temperature, and green rings, dots, and mottling appeared. No virus-free controls reacted in this manner, nor did virus-carrying plants under ordinary greenhouse conditions. However, grafting is believed to be a better

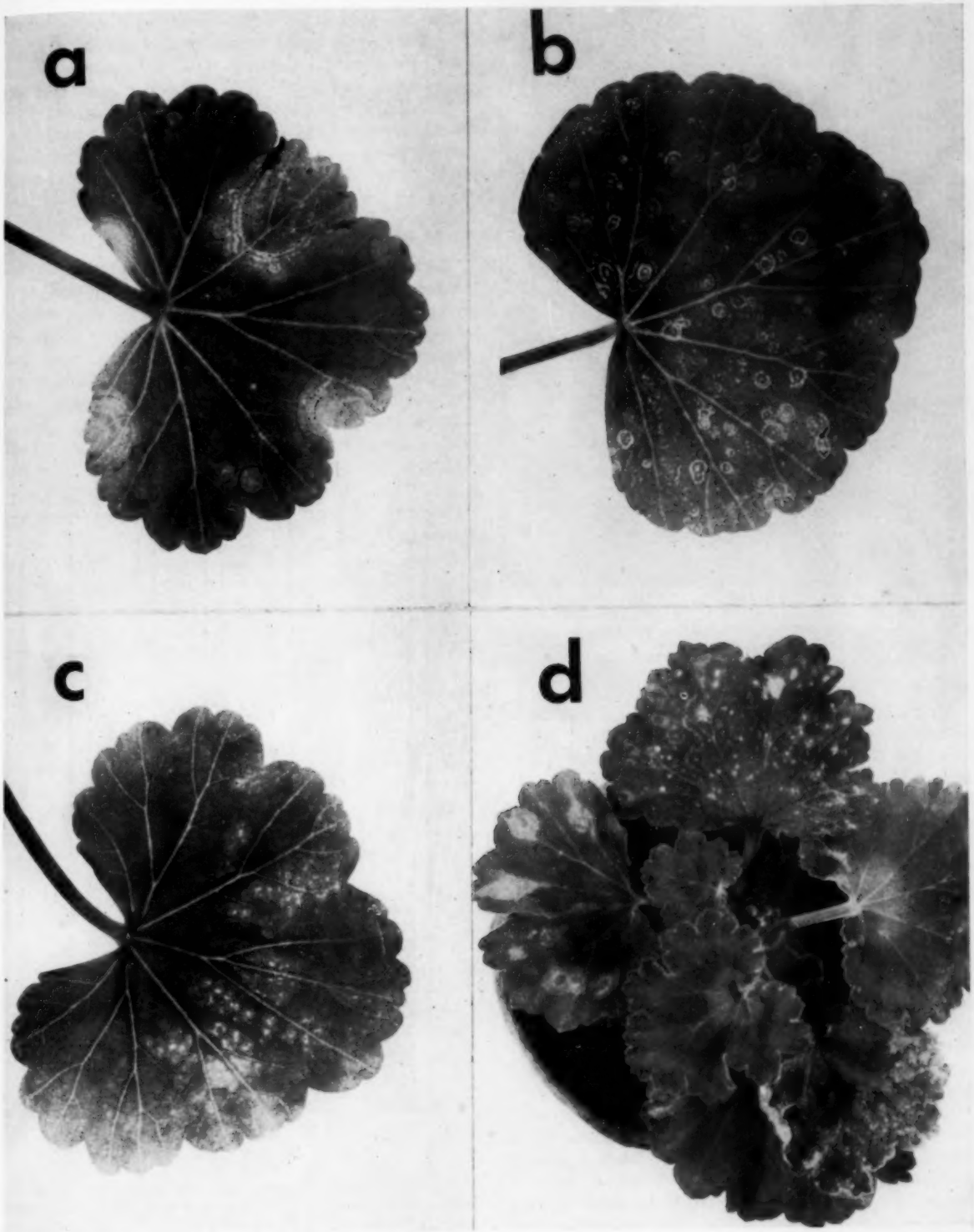


**Fig. 1.** Symptoms of virus infection in pelargonium. Initial symptoms in seedling plants are shown in a, b, and c: **a)** necrotic ringspot, **b)** ringspot, **c)** mottle. **d)** Ringspot and intergrading symptoms found in commercial plantings.

**Fig. 2.** Leaf cupping in a seedling plant grafted with a scion from a symptomless plant. This symptom is thought to be caused by the curly-top virus.

method for detecting infected plants.

Mechanical transmission was tried as a method of detecting latent infections. Sap from leaves, petals, and roots of known virus-carrying pelargonium plants was rubbed on leaves of *Nicotiana glutinosa* L., *Cucumis sativus* L. (var. National Pickling), *Vigna sinensis* Endl., *Gomphrena globosa* L., *Zinnia elegans* Jacq., tomato (vars. Bonny Best and Jefferson), and *P. hortorum seedlings*. No index plant had developed virus symptoms 6 weeks after attempted inoculations.

Dodder (*Cuscuta arvensis* Beyrich), established after some difficulty on pelargoniums, was allowed to grow from known virus-infected plants to tomato, zinia, cucumber, carrot, tobacco, *Geranium maculatum* L., and *P. hortorum* seedlings. No virus symptoms appeared within 6 months, when the plants were discarded.

Several attempts to transmit the diseases by aphids and leafhoppers failed.

*Prevalence of latent viruses in Pelargonium varieties.*—Prevalence of symptomless infection was determined by an indexing survey of 6 varieties from commercial sources, consisting of 100 plants each of Ricard, Radio Red, Olympic Red, Irvington Beauty, Enchantress Supreme, and Fiat. All plants were free of virus symptoms and bacterial stem rot symptoms at assay. The survey showed that at least 62% of all plants carried latent but detectable viruses (Fig. 3). Except for Enchantress Supreme, some plants of all varieties appeared free of latent infections (Fig. 3). However, it is not now possible to conclude that all plants reacting negatively were actually free of virus. For example, a few Fiat plants that appeared virus-free in the assays showed virus-like symptoms several years later, and in at least one case a graft transfer to seedlings was successful from such plants. This could have resulted from spread in the greenhouse, but such dissemination is unknown. In addition, many stock plants that reacted positively in the assays were observed for several years, and in almost every case they later showed symptoms similar to those described by McWhorter (5).

An attempt was made to determine the relative prevalence of the various latent conditions in standard varieties. Ringspot, necrotic ringspot, and crinkle were most common. For example, grafts made on seedlings showed that 69% of the Ricard plants had apparent virus infection. Of the Ricard plants reacting positively in assay, 32 in 100 gave ringspot, 12 gave necrotic ringspot, and 25 gave crinkle. Mottle and mosaic were common in some varieties. Leaf cupping was rare. A ringspot, presumably of virus

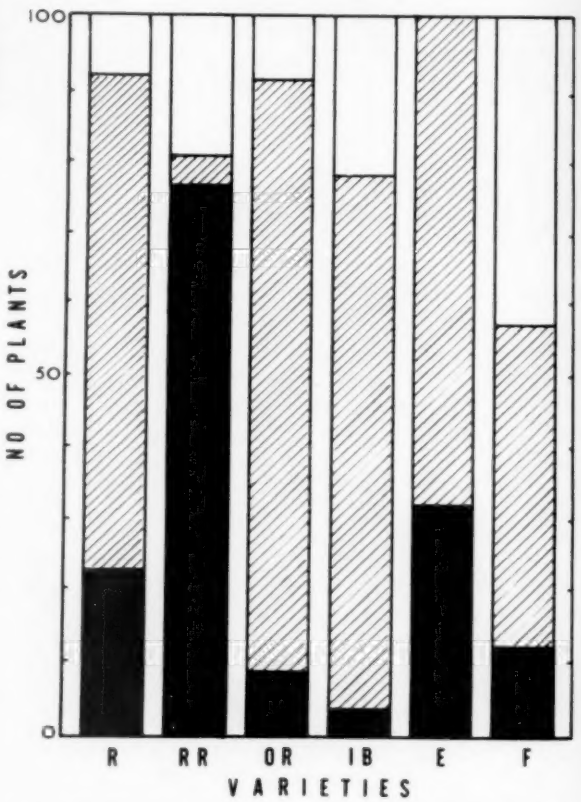


**Fig. 3.** Numbers of plants carrying latent virus (shaded) infections in pelargonium varieties Ricard **(R)**, Radio Red **(RR)**, Olympic Red **(OR)**, Irvington Beauty **(IB)**, Enchantress Supreme **(E)**, and Fiat **(F)** from commercial sources. Black parts of the graph represent plants lost to *Xanthomonas pelargonii*.

TABLE 1.—*Stem rot development in Pelargonium hortorum seedlings as affected by virus infection*

| Virus condition | Total no. plants | % plants receiving each stem rot rating[a] | | | |
|---|---|---|---|---|---|
| | | None | Slight | Medium | Severe |
| Ringspot | 130 | 19 | 39 | 23 | 19 |
| Necrotic ringspot | 22 | 9 | 27 | 18 | 46 |
| Mottle | 44 | 14 | 18 | 27 | 41 |
| Crinkle | 36 | 6 | 11 | 28 | 55 |
| Virus-free | 102 | 33 | 51 | 8 | 8 |

[a] Stem rot symptoms were recorded 50 days after inoculation with *Xanthomonas pelargonii*. Night temperature was 16°C.

origin, was observed on several occasions in commercial ranges, but the symptom (Fig. 1-d was not the same as that seen as an initial reaction in seedlings.

*Variable responses following grafting.*—A quantitatively different response often resulted when seedlings were grafted with different scions from the same plant. Scions from lower stem portions caused more pronounced symptoms in seedlings stocks than did scions from the upper stem. Seedling understocks grafted with 2 or more scions had more pronounced initial symptoms than did those grafted with one scion. Cuttings from lower stems of known virus-diseased plants usually showed virus symptoms after root formation, when shoot growth was resumed, regardless of season. At the same time, cuttings from the upper stem showed symptoms less distinctly and less frequently than did cuttings from lower stem portions. This agrees with the observations of McWhorter (5), and is believed to reflect differences in virus concentrations, which in turn may cause differences in initial reactions.

*Effect of virus infections on bacterial stem rot development.*—Pelargoniums infected with viruses normally show symptoms only in late winter and spring (2, 5), and harm to growth or production is hard to determine. However, many factors unfavorable to a host will promote bacterial stem rot development (4), so the possibility was considered of an interaction between bacterial stem rot and virus infection. In one experiment, *P. hortorum* seedlings were grafted with scions from virus-free plants, and others were grafted with scions from known virus-diseased plants. Virus infection was determined by symptoms in seedlings 40 days after grafting, and all those without positive symptoms were discarded. Stems were then inoculated with *X. pelargonii*, and plants were kept at 16°C night temperature. Stem rot developed most rapidly in plants with crinkle, mottle, and necrotic ringspot, more slowly in plants with ringspot, and slowest in virus-free seedlings (Table 1).

Rooted cuttings from virus-free and known virus-infected plants, var. Ricard, were inoculated with *X. pelargonii*, and stem rot development at 16°C night temperature was compared. Stem rot again developed more slowly in virus-free than in known virus-carrying plants. Of the virus-infected plants, disease developed most rapidly in plants with mottle and necrotic ringspot.

Pelargonium seedlings or plants of the variety Ricard were used in these experiments. Both are more resistant to stem rot than are many standard varieties (4). This, combined with the relatively low night temperatures, probably accounts for the low disease incidences reported in Tables 1 and 2. The results must be interpreted with caution, because of such factors and the small numbers of plants used. Nevertheless, it is believed that the data indicate an effect of some viruses on resistance to bacterial stem rot.

*Heat therapy.*—The possibility of eliminating viruses from pelargonium with high temperature was tested in one experiment. Six plants with ringspot, 10 with necrotic ringspot, 8 with mottle, and 10 virus-free plants were kept at 40°C for 3 days, and 7, 11, 13, and 10 plants, in the same order, were kept at 40°C for 7 days. All plants were then returned to the greenhouse. Like numbers were kept the whole time, as controls, under greenhouse conditions at 20°C night temperature. Scions from each plant, both heat-treated and controls, were veneer-grafted to *P. hortorum* seedlings before and after treatment. The heat treatments did not eliminate the virus from plants. There was a slight reduction in numbers of heat-treated plants from which the virus was recovered, but so few plants were used that this was not considered significant.

DISCUSSION.—When pelargoniums were produced commercially from locally grown propagating stocks, diseases were local or of minor significance. Recently, more and more greenhouse flower growers in the Midwest and East have turned to California for plants and cuttings to root or finish to marketable size. Apparently, California stock is used in the Pacific Northwest also (5). Pelargonium production all over the country is now faced with serious disease problems, probably because pelargoniums are grown in California under conditions that favor spread of bacterial blight and viruses. Although the conditions presumed to be of virus origin have not been observed to spread in the greenhouse, they are known to spread in plantings out-of-doors (2). Unknown insect vectors are evidently involved. Leafhoppers and other insects are common in California fields, and pelargoniums are said to harbor aphids during cool weather. Thus the

TABLE 2.—*Stem rot development in rooted cuttings (var. Ricard) as affected by virus infections*

| Virus condition | Total no. plants | No. plants receiving each stem rot rating[a] | | | |
|---|---|---|---|---|---|
| | | None | Slight | Medium | Severe |
| Ringspot | 25 | 9 | 11 | 3 | 2 |
| Necrotic ringspot | 25 | 5 | 2 | 10 | 8 |
| Mottle | 25 | 2 | 4 | 7 | 12 |
| Virus-free | 25 | 18 | 5 | 1 | 1 |

[a] Stem rot symptoms were recorded 50 days after inoculation with *Xanthomonas pelargonii*. Night temperature was 16°C.

widespread occurrence of certain virus diseases and of bacterial stem rot is probably associated with the fact that a small area in California supplies most of the pelargonium stocks for the whole country.

Latent virus infections can be detected by removing a scion from the plant in question and grafting it to a virus-free seedling stock. Definite symptoms often develop in the leaves of the understock. There is no assurance that this method will detect all viruses, but it made possible a survey that showed a high incidence of latent infections in certain stocks. Some limited unpublished data indicate that the method may be improved for certain viruses by selecting sensitive seedling clones. Perhaps it could be adapted for screening commercial stocks to eliminate viruses, as has been done with chrysanthemums. Grafting assays agreed fairly well with other observations that indicate the presence of viruses. In most cases plants that reacted positively in the assay developed symptoms some months or years later. Such plants also developed peculiar leaf markings under conditions of low light and low temperature.

The common reactions in seedlings after grafting with scions from plants of standard varieties were ringspot, necrotic ringspot, crinkle, mottle, and mosaic —and sometimes leaf cupping. The various symptoms may intergrade, and may be manifestations of one virus or of multiple infections, and the possibility was not ruled out that different viruses may cause similar symptoms. At any rate, grafting from any one plant caused qualitatively uniform responses in seedlings from one assay to another. To determine whether single or multiple infections are involved, a means must be found to transmit viruses from pelargonium to other hosts.

The significance of the latent conditions is hard to determine. Certain of them, especially the one known as crinkle, can become severe enough to destroy the commercial value of plants. The leaf spotting described by McWhorter (5) and some ringspot conditions can seriously impair the growth and appearance of pot plants, though the plants may carry these and other conditions in a latent form with no apparent ill effects. However, the possibility that such plants are less resistant to bacterial stem rot makes even symptomless infections of some concern. There is still the possibility that pelargoniums may serve as virus reservoirs from which other crops are infected.

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# SUBSPECIFIC IDENTITY OF THE STEM RUST FUNGUS OF MERION BLUEGRASS[1]

Michael P. Britton and George B. Cummins [2]

SUMMARY

In studies to determine the subspecific identity of the stem rust fungus (*Puccinia graminis* Pers.) of the Merion variety of bluegrass (*Poa pratensis* L.), isolates were capable of sporulating on 39 species in 15 genera in 5 tribes of the Gramineae, including *Agrostis palustris* Huds., but did not infect species of *Avena, Hordeum, Phleum, Triticum,* and others. The percentage of urediospores with 3 germ pores was nearly identical in collections from Merion bluegrass (71.2%), *Agrostis alba* L. (72.0%), and American *Poa* spp. (75.0%), but different in collections from *Phleum pratense* L. (47.0%), European *Poa* spp. (32.8%), *Avena sativa* L. (1.6%), *Triticum aestivum* L. (1.0%), and *Secale cereale* L. (0.2%). It is concluded that the stem rust of Merion bluegrass is incited by a race of *P. graminis* f. sp. *agrostis.*

The Merion variety of bluegrass (*Poa pratensis* L.) was released in 1950, by the United States Golf Association Green Section in cooperation with the U. S. Department of Agriculture. Extensive testing at Arlington Farm, Virginia, and at Beltsville, Maryland, from 1936 to 1950 showed that, for turf, Merion was superior to ordinary Kentucky bluegrass. However, the desirability of Merion was in part nullified in 1953, when its extreme susceptibility to stem rust (*Puccinia graminis* Pers.) was noted (3, 4, 5). By 1955 the disease had been reported in epiphytotic severity on Merion bluegrass plantings from Rhode Island to California. This sudden increase in severity indicated that the pathogen had probably been present and widespread before release of the Merion selection. Since isolates of *P. graminis* from *Poa* spp. in the U. S. had previously been identified as *P. graminis* Pers. f. sp. *poae* Eriks. & E. Henn. (6) and as physiologic races of *P. graminis* Pers. f. sp. *avenae* Eriks. & E. Henn. (1), the subspecific identity of the fungus on Merion bluegrass became a question. Accordingly, experiments were begun to determine the approximate range of grasses that might serve as host plants of the rust fungus from Merion bluegrass. It was assumed that comparing the results with data already available on the *formae speciales* of *P. graminis* would provide a satisfactory basis for identification. However, preliminary observations of the urediospores of *P. graminis* from Merion bluegrass indicated that there might be a higher proportion of spores with 3 germ pores than was thought to be typical of the species. Hence, a study was also conducted to determine the possible significance of germ pore number in recognizing the *formae speciales* of *P. graminis.*

EXPERIMENTAL METHODS AND RESULTS.—The potential host range of *P. graminis* was determined by greenhouse inoculation experiments with 2 single-pustule isolates from Merion bluegrass. Both cultures produced identical infection types on clonal lines of *Poa pratensis* and *Poa compressa.* Infection types were recorded 14–16 days after inoculation, according to the scale described by Stakman et al (7). Only species on which the rust was able to sporulate were considered to be hosts of the fungus—39 species, distributed in 15 genera of 5 tribes of the Gramineae (Table 1). It is doubtful whether species showing only resistant reactions (1 or 2) would serve as natural hosts. However, the plant population tested within most species was rather narrow, and fully susceptible plants may exist in species that gave resistant reactions. Such a possibility is indicated by the variability of reaction recorded among the 41 selections of *Poa pratensis* that were tested.

No infection was visible on the following species: *Agrostis alba* L., *Avena byzantina* C. Koch. *A. fatua* L., *A. sativa* L., *A. strigosa* Schreb., *Bromus inermis* Leyss., *B. marginatus* Nees, *B. tomentellus* Boiss., *Festuca arundinacea* Schreb., *F. ovina* L., *F. rubra* L., *Holcus lanatus* L., *Hordeum hystrix* Roth, *H. jubatum* L., *Lolium perenne* L., *Phleum pratense* L., *Poa nemoralis* L., *Stipa columbiana* Macoun, *Triticum aestivum* L., *T. compactum* Host., *T. dicoccum* Schrank, *T. durum* Desf., and *T. monococcum* L.

Since the potential host range of *P. graminis* from Merion bluegrass is rather extensive, one would expect that some of these hosts might be susceptible to other *formae speciales* of *P. graminis.* This was substantiated by a comparison of the host range with the published host ranges of other *formae speciales* (2). The isolate from Merion has 6 fully susceptible host species in common with *P. graminis* f. sp. *avenae.* None of the common hosts are in the genus *Avena.* The Merion rust fungus has 4 hosts in common with *P. graminis* var. *phlei-pratensis* (Eriks. & E. Henn.) Stakm. & Piemeisel. But *Phleum pratense* is immune to the Merion rust. The Merion rust fungus has 4 common hosts with *P. graminis* f. sp. *agrostis* Eriks., and susceptible reactions are produced on all of these hosts by both rusts. One of the hosts is *Agrostis palustris.*

In the study of the germ pores, urediospores of *P. graminis* were examined from 5 or more collections

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of each grass species that was considered a representative host for each of the *formae speciales* occurring in the United States. Rust specimens from species of *Poa* collected in Europe were also studied. Most of the material examined was obtained from specimens in the Arthur Herbarium, Purdue University. As far as possible, the specimens selected for study had been collected in widely separated geographic areas and in different years. It was thought that specimens separated in both place and time would give a better measure of the stability of the germ pore characters than would collections made in 1 locality on 1 date. Chi-square tests for homogeneity were applied to the collections from each host species.

Observation of the germ pores was facilitated by the clearing action resulting from: 1) leaving the spores in a water mount for 1 hour, 2) replacing the water with a saturated solution of chloral hydrate, and 3) making the counts after the spores had remained in the chloral hydrate solution for 12 hours or more. A microscope with a mechanical stage was used.

The results are presented graphically in Fig. 1. Chi-square tests for homogeneity indicate that the frequency of urediospores with 3, 4, and 5 germ pores

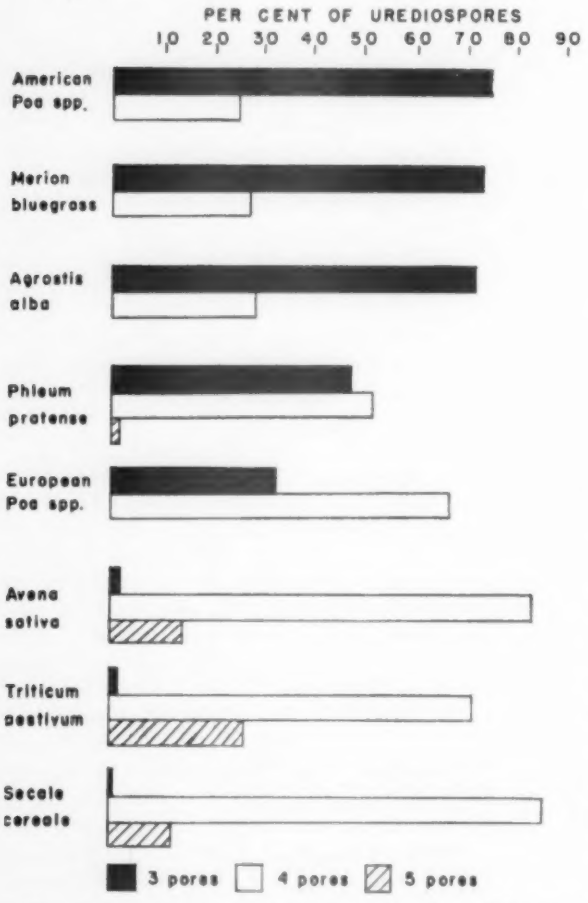


**Fig. 1.** Per cent of urediospores with 3, 4, and 5 pores in 5 different collections from each grass host.

TABLE 1.—*Infection types produced on grasses infected by Puccinia graminis isolated from Merion bluegrass*

| Tribes and species | Infection types |
|---|---|
| Festuceae | |
| *Dactylis glomerata* L. | 0–3 |
| *Festuca idahoensis* Elmer | 4 |
| *Poa alpina* L. | 3–4 |
| *P. ampla* Merr. | 2–4 |
| *P. annua* L. | 4 |
| *P. arachnifera* Torr. | 1 |
| *P. arctica* R. Br. | 2–4 |
| *P. bulbosa* L. | 4 |
| *P. caespitosa* Forst. | 4 |
| *P. canbyi* (Scribn.) Piper | 1–4 |
| *P. compressa* L. | 0–2 |
| *P. cusickii* Vasey | 3–4 |
| *P. fendleriana* (Steud.) Vasey | 3–4 |
| *P. gracillima* Vasey | 4 |
| *P. interior* Rydb. | 1–3 |
| *P. iridifolia* Hauman | 3 |
| *P. nervosa* (Hook.) Vasey | 3–4 |
| *P. occidentalis* Vasey | 0–2 |
| *P. pratensis* L. | 0–4 |
| *P. reflexa* Vasey et Scribn. | 2–3 |
| *P. rupicola* Nash | 2 |
| *P. scabrella* (Thurb.) Benth. ex Vasey | 2–3 |
| *P. secunda* Presl. | 4 |
| *Puccinellia airoides* (Nutt.) Watts. et Coult. | 4 |
| Hordeae | |
| *Agropyron latiglume* (Scribn. et Smith) Rydb. | 0–1 |
| *A. spicatum* (Pursh) Scribn. et Smith | 1–3 |
| *Elymus canadensis* L. | 0–1 |
| *E. virginicus* L. | 0–1 |
| *Sitanion hystrix* (Nutt.) J. G. Smith | 0–1 |
| Aveneae | |
| *Arrhenatherum elatius* (L.) Presl. | 1–2 |
| *Koeleria cristata* (L.) Pers. | 4 |
| *Trisetum spicatum* (L.) Richt. | 4 |
| Agrostideae | |
| *Agrostis palustris* Huds. | 3 |
| *Alopecurus arundinaceus* Poir. | 3 |
| *A. carolineanus* Walt. | 1–3 |
| *A. pratensis* L. | 3 |
| *Apera interrupta* (L.) Beauv. | 3 |
| *Oryzopsis asperifolia* Michx. | 1 |
| Phalarideae | |
| *Phalaris arundinacea* L. | 1–2 |

may be a relatively constant character within a given *forma specialis* of *P. graminis*, and that differences in germ pore frequencies are significant between *P. graminis* from Merion bluegrass and *P. graminis* from *Phleum pratense*, European species of *Poa*, *Avena sativa*, *Triticum aestivum*, and *Secale cereale*. The rusts from Merion bluegrass and *Agrostis alba* are almost identical in germ pore frequency. Chi-square tests for homogeneity support these conclusions. Moreover, all other American collections of stem rust on species of *Poa* examined had germ pore frequencies characteristic of f. sp. *agrostis*. Among the American collections was a specimen labeled *P. graminis poae* on *Poa compressa*, collected on August 20, 1923, near

Pontiac, Michigan (sent to Dr. J. C. Arthur by Dr. E. C. Stakman), which appears to be the basis of the original report of Stakman and Levine (6) of the occurrence of f. sp *poae* in North America.

Two major groups of *formae speciales* are readily apparent in classification by germ pore number. One group, composed of the *P. graminis* collections from *Avena sativa*, *Triticum aestivum*, and *Secale cereale*, has a relatively high proportion of urediospores with 5 germ pores, and few with 3 germ pores. The second group, having a high frequency of urediospores with 3 germ pores and few or no spores with 5 pores, encompasses the collections from the *Poa* spp., *Phleum pratense* and *Agrostis alba*. In general, the occurrence of 5 germ pores is correlated with the *formae speciales* having the larger urediospores, and the occurrence of 3 germ pores with those having the smaller urediospores.

Discussion.—It would be difficult to assign the rust fungus on Merion bluegrass to a particular *forma specialis* on the basis of the host-range study alone, since it has hosts in common with *P. graminis* f. sp. *avenae*, *P. graminis* f. sp. *agrostis*, *P. graminis* var. *phlei-pratensis*, and *P. graminis* f. sp. *poae*. However, the susceptibility of *Agrostis palustris* and the nearly identical germ pore frequencies of urediospores of *P. graminis* from Merion bluegrass and from *Agrostis alba* do indicate a relationship. On the basis of this evidence it is concluded that *P. graminis* on Merion bluegrass is a physiologic race of *P. graminis* f. sp. *agrostis*. Since the germ pore frequencies encountered in collections of *P. graminis* from *Poa species* collected in the United States are not significantly different from those of stem rust collections from *Agrostis alba*, it is concluded that the rust on these species is also *P. graminis* f. sp. *agrostis*.

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# BACTERIAL RHIZOME ROT OF BANANAS[1]

R. H. Stover

SUMMARY

A rhizome rot of bananas, prevalent in most banana-growing areas, was found to be incited by *Pectobacterium carotovorum* (Jones) Waldee (Syn. *Erwinia carotovora* (Jones) Holland. Newly planted rhizomes and young plants less than 6 months old are frequently destroyed, but the disease is most serious in young banana plantations 8 to 20 months old. Soils previously flood-fallowed for Fusarium wilt control favor development of the disease. Wounding increased disease severity; weekly applications of Agrimycin drench in greenhouse tests failed to control the disease on wounded artificially inoculated rhizomes.

In 1953, Hord and Flippin (4) described the symptoms of a banana disease called rhizome head rot, first observed in 1948, in the Aguan Valley of Honduras. A watery decay of the banana rhizome resulted in the toppling over of the banana plant. Bacteria, but no fungi, were obtained from the rotting rhizomes. Banana root borers, *Cosmopolites sordidus* (Germ.), were frequently associated with the rot. In a later paper, Hord and Flippin (5) showed that banana root borers attacked rhizomes independently of the head rot organism. Wardlaw (9) mentioned a banana disease in Jamaica called "blackhead," which referred to any disease of the rhizome showing superficial or internal brown or dark water-soaked patches. Ashby (1) obtained species of *Thielaviopsis*, *Botryodiplodia*, *Gloeosporium*, and *Verticillium* from "blackhead"-diseased rhizomes, but did not prove the pathogenicity of these fungi. Cobb (2) and, more recently, Loos (7) and Leach (6) found the nematode *Radopholus similis* (Cobb) Thorne associated with a "blackhead" disease in Panama and Jamaica.

It is apparent that 3 groups of organisms are associated with rots or "blackhead" diseases of banana rhizomes: bacteria, fungi, and nematodes. The pathogenicity of the bacteria or fungi obtained from rotting rhizomes has not been reported. This paper describes the isolation, pathogenicity, and taxonomy of the soft rot bacterium responsible for a rhizome rot of Gros Michel bananas in Honduras.

SYMPTOMS.—In the field, symptoms are manifested at 3 stages of growth: 1) rotting of newly planted rhizomes, with resultant failure to sprout; 2) stunting and poor growth of young plants; or 3) toppling over of mature plants bearing fruit.

Regions of dark- to pale-brown water-soaked tissue develop from the rhizome surface inward through the cortex and stele (Fig. 1), occasionally from only one portion of the rhizome. The rhizome eventually becomes internally pocketed with dark-brown or yellow water-soaked areas with dark peripheral rings (Fig. 1, 2). The interiors frequently decay, leaving cavities surrounded by dark, spongy matter. Many of the developing spots are not contiguous, indicating that the pathogen moves from one area to another before setting up a new center of tissue breakdown. In potted plants in the greenhouse, the rot frequently begins in the cortex, in association with old leaf sheath remnants attached to the rhizome near the central growing point.

**Fig. 1.** Cross section of mature rhizome naturally infected with *Pectobacterium carotovorum*. **Fig. 2.** Tangential section of base of fallen pseudostem, with portion of rotted rhizome attached. The rot has passed up into the base of the pseudostem leaf sheaths.

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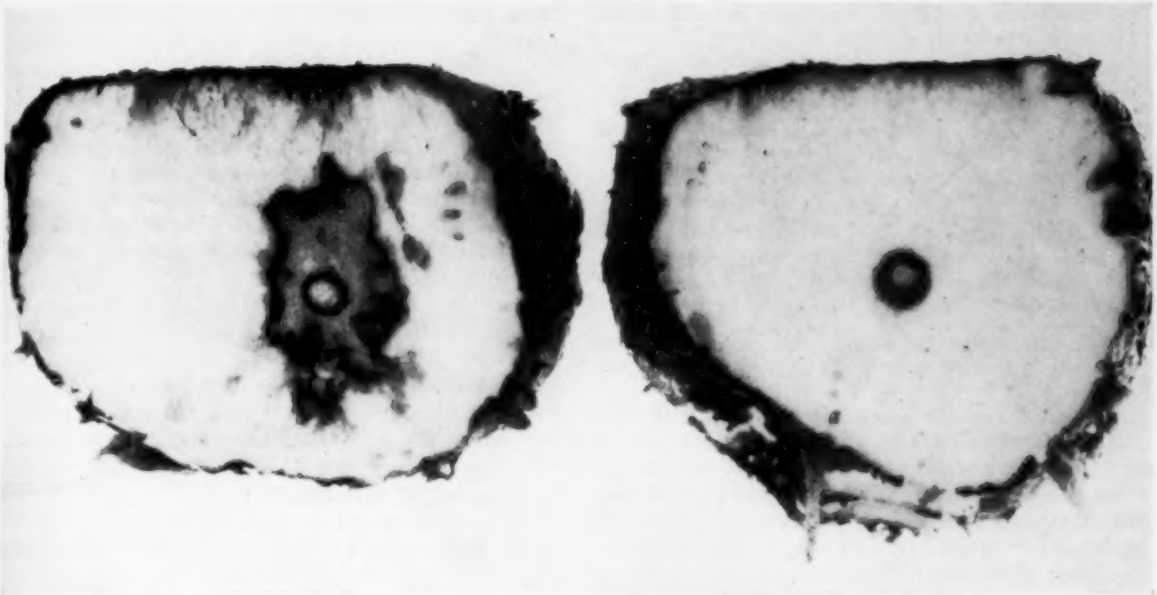

**Fig. 3.** Artificial inoculation of healthy rhizome with *Pectobacterium carotovorum*. A hole of ⅜-in. diameter and 1½-in. depth was punched in each rhizome with a cork borer. A 2-mm square piece of a nutrient agar slant culture of *P. carotovorum* was placed in the bottom of the hole of the rhizome on the left, and agar only in the rhizome on the right. The plug of rhizome tissue was reinserted and the wound coated with paraffin before the rhizome was planted in sterile soil.

As the rot progresses, young plants become stunted and yellow and are easily pushed over. Mature plants seldom exhibit outward symptoms but are easily blown or pushed over, particularly when the fruit becomes heavy. When the pseudostem of a mature diseased plant falls over, the rhizome splits and a portion of the infected tissue adheres to the pseudostem; the remainder of the rhizome stays anchored by the roots. In severe cases the young rhizomes of the suckers emerging around the mature rhizome are infected from the mature plant. However, the suckers frequently remain healthy, particularly in mild cases of disease. It is noteworthy that the roots are not attacked directly, but are destroyed through rotting of the rhizome. The hollow pockets of rot in the outer cortex are usually colonized by ants. Disease symptoms above ground appear about 8–10 weeks after inoculated rhizomes are planted in pots.

Distribution and development of rhizome rot.—In Honduras, the disease is most serious on Gros Michel bananas in areas previously flood-fallowed 4–6 months, and on bananas in methyl-bromide-fumigated or steam-sterilized soil in the greenhouse. An incidence of 10–15% is common in the field, and up to 50% of the banana mats are occasionally affected in some areas. Losses are greatest during the first 8–20 months after planting; thereafter the incidence usually declines, becoming minor as the plantation ages. Newly planted rhizomes and plants less than 6 months old are frequently destroyed. Rhizome rot often interferes with the interpretation of experiments on other diseases in both greenhouse and field. No seasonal trend in incidence is apparent, nor does the disease occur consistently in all areas. Rhizomes rots in all the major banana-growing areas of Central America and the Caribbean islands are possibly a single disease.

Pathogenicity.—Microscopic examination of adjacent diseased and healthy tissue revealed neither fungi nor nematodes, but bacteria were abundant in the cells and intercellular spaces. Nutrient agar plates streaked with macerated diseased tissue yielded almost pure cultures of a bacterium. The bacterium was grown 4 days either in nutrient broth in 25-ml flasks or on nutrient agar slants. To determine the pathogenicity of the bacterium, banana rhizomes were washed and surfaces disinfected 3 minutes in 5% Clorox, a localized area was flamed, and a ⅜-in. plug of tissue was removed to a depth of 1–1½ in. with a sterile cork borer. A piece of a nutrient agar culture of the bacterium 3–4 mm square was placed at the base of the wound, and the plug was reinserted. The wound was then coated with paraffin, and the rhizomes were planted in sterilized soil or vermiculite. In the check treatments, a piece of nutrient agar alone was inserted in the wound.

The rhizomes were removed 25 days after planting, cut open and examined. Six in 10 of the inoculated rhizomes exhibited symptoms typical of rhizome rot around the area of inoculation (Fig. 3). In most cases the rot had spread about 1½ in. from the point of inoculation. Isolations from the outer periphery of the rotted zone yielded pure cultures of the bacterium inoculated.

TABLE 1.—*Effect of different treatments on area of rhizome invaded by bacterial rhizome rot of bananas*

| Rhizome treatment | No. of rhizomes having rot in varied percentages | | | | |
|---|---|---|---|---|---|
| | 0 | 5–10 | 11–25 | 26–50 | 51–100 |
| Bacterial dip | 6 | 5 | 1 | 0 | 2 |
| Bacterial dip + wounding | 0 | 0 | 3 | 2 | 10 |
| Bacterial dip + wounding + weekly streptomycin drenches | 0 | 3 | 1 | 1 | 10 |
| Check (wounded only)[a] | 13 | 2 | 0 | 0 | 0 |
| Check (no treatment) | 12 | 1 | 2 | 0 | 0 |

[a] Wounding consisted of 3–4 cuts with a sharp knife 4 in. long and ¼–½ in. deep, immediately before the rhizome was dipped in water or water plus a bacterial suspension.

EFFECT OF AGRIMYCIN ON DISEASE CONTROL.—A series of experiments were undertaken to test the effects of wounding and Agrimycin. Washed rhizomes, with or without wounding, were dipped in 200 ml of a 4-day-old nutrient broth culture of the bacterium suspended in 5.7 liters of tap water. The rhizomes were then planted in 12-in. pots containing soil fumigated with methyl bromide. In one series of treatments the plants were first dipped 20–30 seconds in Agrimycin, and 1 qt. of Agrimycin suspension was applied weekly to the base of each plant. Agrimycin, which contains 15% streptomycin and 1.5% terramycin, was used at the rate yielding 100 ppm of streptomycin. Wounding greatly increased the incidence of rhizome rot; Agrimycin had no effect (Table 1).

TAXONOMIC STUDIES OF THE PATHOGEN.—The bacterium is a motile gram-negative rod that forms dull-greyish-white colonies on nutrient agar. Potato and onion slices and whole green tomatoes were rotted within 48 hours of inoculation. Salicin, glucose, lactose, and sucrose at 1% concentrations were fermented in 4 days, in a peptone-free medium with production of acid and small amounts of gas. Maltose was not fermented. Liquid depressions were formed on pectate-gel medium (3), indicating the presence of pectic enzymes. It was concluded that the morphologic and physiologic characteristics of the bacterium as repeatedly isolated were identical to those of *Pectobacterium carotovorum* (Jones) Waldee, the incitant of numerous soft rot diseases of storage tissues. The species name *P. carotovorum* as defined by Waldee and cited in Dowson (3) is used in preference to that of *Erwinia carotovora* (Jones) Holland.

CONTROL.—No control has been developed. Disease intensity in the field declines with time, indicating that biological factors are operative in checking disease spread.

DISCUSSION.—Since *P. carotovorum* attacks the rhizome but not the roots, it is easily distinguished from the "blackhead" disease of rhizomes induced by *Radopholus similis* (2, 6, 7). These nematodes were not observed in the tissue, and were absent or rare where the incidence of rhizome rot was highest (8).

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# PURIFICATION, PROPERTIES, AND SEROLOGY OF CUCUMBER MOSAIC VIRUS[1]

J. A. Tomlinson,[2] R. J. Shepherd, and J. C. Walker

SUMMARY

A method of purifying CMV (cucumber mosaic virus) was found that involves homogenizing infected tissue in strong buffer containing a reducing agent. The filtrate of the homogenate was clarified with n-butanol, and the virus was concentrated and partially purified by differential centrifugation. Acid precipitation and further differential centrifugation of the preparation yielded a purified virus solution, which was found to contain an nucleoprotein with a sedimentation constant of 101 S. Electron micrographs showed that air-dried purified virus solutions contained spherical particles with a mean diameter of 40 mμ. An antiserum to the virus with a titer of 1/1024 was prepared from partially purified preparations. Tests showed that it could be used for detecting CMV in unpurified sap.

From experiments with the sap of cucumber plants showing mosaic symptoms Doolittle (4) concluded that the disease was caused by a virus. It has since been shown that CMV (cucumber mosaic virus) can infect a wide range of plants and has widely differing strains. The virus loses its infectivity in plant sap if heated 10 minutes to about 60°C, stored 2–3 days at room temperature, or diluted above $10^{-3}$. It thus appears that CMV occurs in a low concentration in infected plants and is unstable *in vitro*. In previous investigations of strain relationships of CMV, cross-protection tests have been used because of the difficulties of preparing antisera of high titer. Opinions differ on the shape of the virus. Some workers (2, 6) have suggested that it is rod-shaped, whereas recent work (5, 11) indicates that it is a spherical particle.

The present investigation was conducted to find a method of purifying CMV, to study its properties and morphology, and to develop an antiserum.

MATERIALS AND METHODS.—The virus used was the Y strain of CMV, used previously by Sill et al (11). This strain was cultured in cucumber (*Cucumis sativus* L.) and tobacco (*Nicotiana tabacum* L. var. Havana 425), thus enabling the detection of contamination with tobacco mosaic virus, which causes local lesions in this tobacco. Infected cucumber corolla, used in the purification studies at first, was obtainable only in small amounts, so leaves of infected tobacco were used later.

Infected tissue was homogenized in buffer in a Waring Blendor for 2–3 minutes. The virus solutions were assayed by inoculating carborundum-dusted leaves of cowpea (*Vigna sinensis* Endl. var. Black) with a cheesecloth pad soaked in the solution to be tested. Inoculated cowpea leaves usually developed necrotic local lesions within 30 hours. Purification work was done in a cold room at 3°C. The virus solutions were centrifuged in a refrigerated Spinco model L ultracentrifuge.

Antisera were prepared by injecting rabbits with partially purified virus preparations. The rabbits were first bled to obtain normal serum and then given 6 intravenous injections of 4 ml at 4–5-day intervals. The rabbits were bled at 2–3-day intervals, beginning 6 days after the last injection, and the serum was separated and stored at –10°C. Tests were made with the virus preparations at various dilutions, and with twofold dilutions of the antiserum. One ml each of diluted antiserum and the virus preparation were mixed in glass tubes of 7-mm diameter, in a water bath at 37°C with the fluid column half immersed so that convection currents gave continuous mixing. In addition, ring tests were made by adding 0.2 ml of varying dilutions of the CMV antiserum in the bottom of glass tubes of 2–3 mm diameter, and a similar volume of the virus preparation to be tested was carefully layered over the serum without mixing. After 30 minutes at room temperature the tubes were examined under a bright light in a dark room, and the amount of precipitate that had developed at the interface in the different tubes was scored. Normal serum and preparations of healthy leaf were used as controls.

HOST-VIRUS STUDIES.—Tests were made to determine what part of the cucumber plant yielded an extract containing the highest concentration of active virus, and the time after inoculation when the virus reached the highest concentration in tobacco leaves.

Various parts of infected cucumber plants were homogenized, and the extracts obtained were assayed on cowpea. The results confirmed earlier findings of Sill and Walker (10) that CMV occurs at a relatively high concentration in cucumber corolla.

The multiplication of CMV in tobacco was studied by determining at daily intervals the concentration of virus in the inoculated leaves as measured by infectivity tests on cowpea. Uniform-sized tobacco plants were inoculated with infected cucumber corolla sap. These plants were placed in a large box lighted by 16 40-watt fluorescent tubes about 30 in. above the plants. Air from outside the box was blown over the plants, which were kept at about 25°C. At each assay, 2 leaf disks, 1 cm in diameter, were cut from each inoculated leaf. The disks were homogenized and the filtered sap was assayed on cowpea, undiluted

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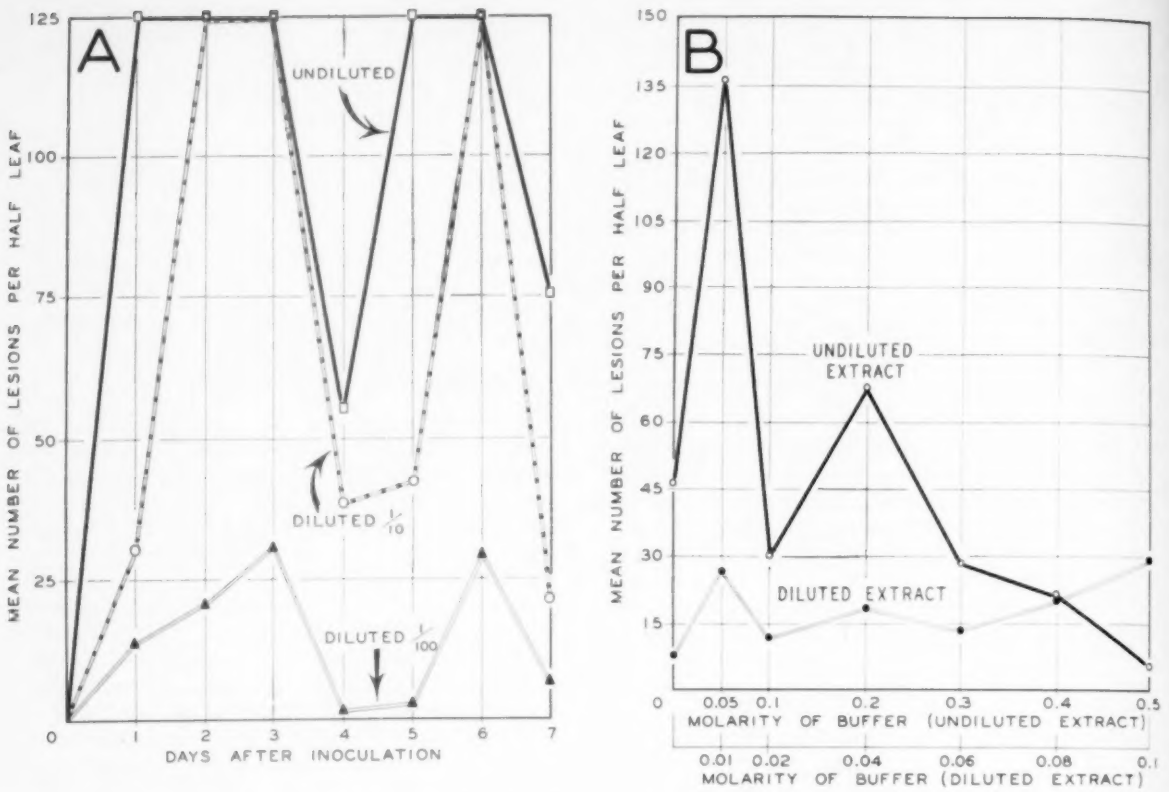


**Fig. 1.** Concentration of cucumber mosaic virus in tobacco and effect of buffer molarity on the infectivity of the virus. **A)** Concentration of the virus in undiluted and diluted extracts of inoculated leaves of tobacco var. Havana 425 at different time intervals after inoculation. **B)** Effect of buffer molarity on infectivity of the virus in an extract from cucumber corolla, undiluted and diluted 1/5.

and diluted 1/10 and 1/100 with *0.05M* potassium-phosphate buffer at pH 7.5. The cowpea plants used for assaying the saps were grown in the same light box.

Preliminary experiments indicated that it was necessary to use an inoculum with a very high titer in order to secure a rapid development of virus in inoculated tobacco leaves. The inoculum used, therefore, consisted of extracts from infected cucumber corollas, or of a partially purified solution of virus from inoculated tobacco leaves. The fluctuation in virus concentration in inoculated tobacco leaves in a typical experiment is shown in Fig. 1A. It is to be seen that in undiluted and diluted extracts a maximum concentration was reached on the third day. In the purification studies, therefore, the leaves of tobacco plants were harvested 3 days after inoculation.

PURIFICATION OF THE VIRUS.—*Buffer concentration.*—Tests were made to determine relative amounts of virus that could be extracted when infected corolla was homogenized in a range of potassium phosphate buffer concentrations at pH 7.5. Corolla samples of 4 g each were homogenized in 16-ml volumes of distilled water or buffers of varying molarities. The macerated samples were filtered, and the infectivity of the undiluted and diluted filtrates was assayed on cowpea (Fig. 1B). Yields of virus, as measured by local lesions, were greatest and least in the undiluted filtrates, respectively, in 0.05 and *0.5M* buffer. When diluted 1/5 in distilled water, however, the latter buffer extract was more infectious than before dilution and yielded the most virus of the diluted series. This indicated that the strong buffer contained the most virus, but infection was inhibited unless the buffer was diluted.

*Presence of reducing agent.*—After filtering the homogenates of infected cucumber corolla or tobacco leaf and *0.5M* phosphate buffer, the filtrates slowly became dark brown, presumably from oxidation. In some tests this reaction appeared to result in a loss in infectivity of the filtrates. The formation of these brown components was largely prevented when 0.1% thioglycollic acid was added to the buffer. It was routinely used, therefore, in all purification work.

*Sap clarification and ultracentrifugation.*—Early attempts to clarify buffered extracts of CMV-infected corolla by centrifugation for 10 minutes at 15,000 rpm resulted in an almost complete loss of the virus from the supernatant. This suggested that the virus was bound to particulate host material that sedimented rapidly when centrifuged. Shepherd and Pound (9) obtained similar results with turnip mosaic virus in

buffered extracts of several hosts. They found, however, that no virus loss occurred in similar extracts treated with n-butanol. A method was therefore tested of using n-butanol in clarifying the filtered homogenate of infected corolla and *0.5M* potassium phosphate buffer (pH 7.5).

In a test, 40 g of corolla and 160 ml of buffer were homogenized. Butanol was added to 5 aliquots of the filtrate to give concentrations of 0, 5, 7, 8 and 9%. The butanol was added drop-wise, and the filtrates were stirred for 30 minutes in corked flasks. Heavy precipitates were formed in filtrates containing 8 and 9% butanol. The solutions were centrifuged 10 minutes at 5000 rpm, the residues discarded, and the supernatants filtered through glass wool. Samples of the filtrates were dialyzed 16 hours against *0.05M* buffer at 3°C, and centrifuged 10 minutes at 5000 rpm, and the supernatants assayed (Table 1). The remainder of the undialyzed supernatants was centrifuged 2 hours at 30,000 rpm to pellet the virus.

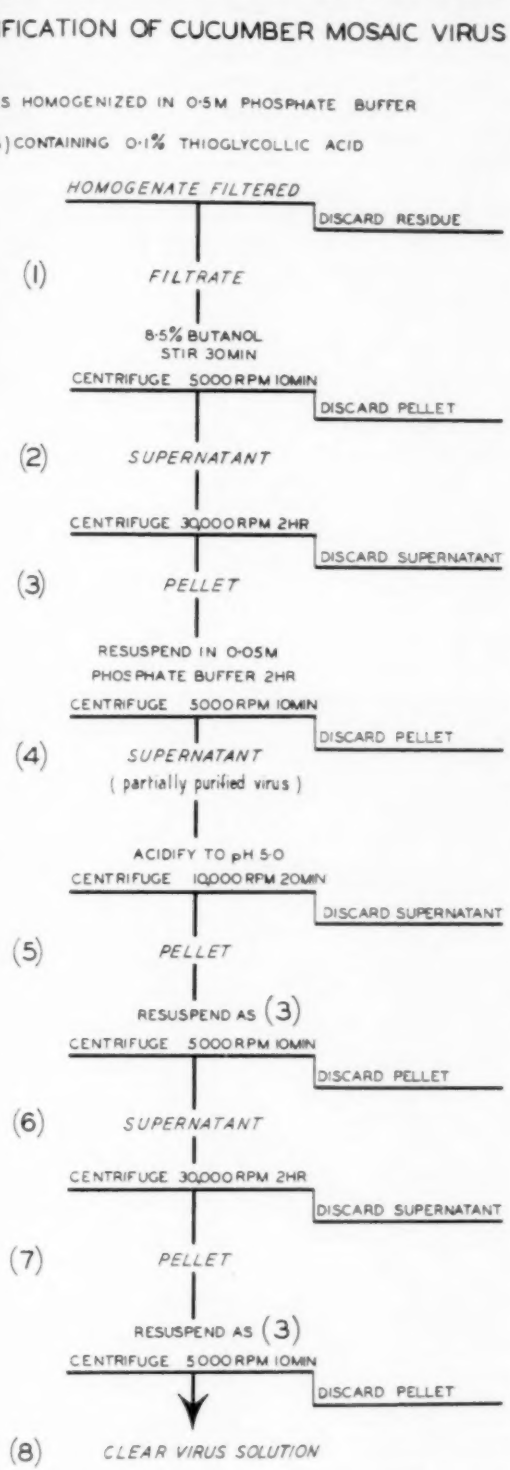


**Fig. 2.** Outline of procedure used for the purification of cucumber mosaic virus.

TABLE 1.—*The concentration of CMV in cucumber corolla after homogenizing in buffer and treatment with n-butanol*

| % butanol added | Degree of clarification[a] | Mean no. local lesions per cowpea half-leaf: Dialyzed virus, Undiluted | Dialyzed virus, Diluted 1/5 | Resuspended virus, Undiluted | Resuspended virus, Diluted 1/5 |
|---|---|---|---|---|---|
| 0 | none | 67 | 1 | 205 | 13 |
| 5 | none | 54 | 1 | 248 | 9 |
| 7 | slight | 92 | 3 | 218 | 14 |
| 8 | clarified | 94 | 5 | 257 | 12 |
| 9 | clarified | 23 | 0 | 121 | 0 |

[a] Degree of clarification of butanol-treated homogenates after centrifugation for 10 minutes at 5000 rpm.

After centrifugation, the supernatants were discarded and the pellets were each resuspended with a mechanical shaker for 2 hours in 1.0 ml of *0.05M* buffer. After resuspension, the virus solutions were centrifuged 10 minutes at 5000 rpm, the pellets discarded, and the supernatants assayed (Table 1).

The results showed that the homogenate could be clarified with 8% butanol without loss of infectivity. Other experiments later showed that the best results were obtained with 8.5% butanol. By ultracentrifugation, the virus could be sedimented from the clarified extracts and resuspended in a small volume of buffer.

Similar results were obtained with infected tobacco

TABLE 2.—*Precipitation of CMV in partially purified preparations in relation to pH of preparation*

| pH | Mean no. lesions per cowpea half-leaf: Supernatant[a] | Pellet[b] |
|---|---|---|
| 7.5 (control) | >500 | 0 |
| 6.5 | >500 | 0 |
| 6.0 | 264 | 30 |
| 5.5 | 47 | 205 |
| 5.0 | 0 | >500 |
| 4.5 | 0 | 283 |
| 4.0 | 0 | 56 |

[a] Supernatant after acidification and centrifugation for 20 minutes at 10,000 rpm.

[b] The pellet resulting from the above centrifugation, resuspended in *0.05M* buffer and made up to a volume equal to that of the supernatants.

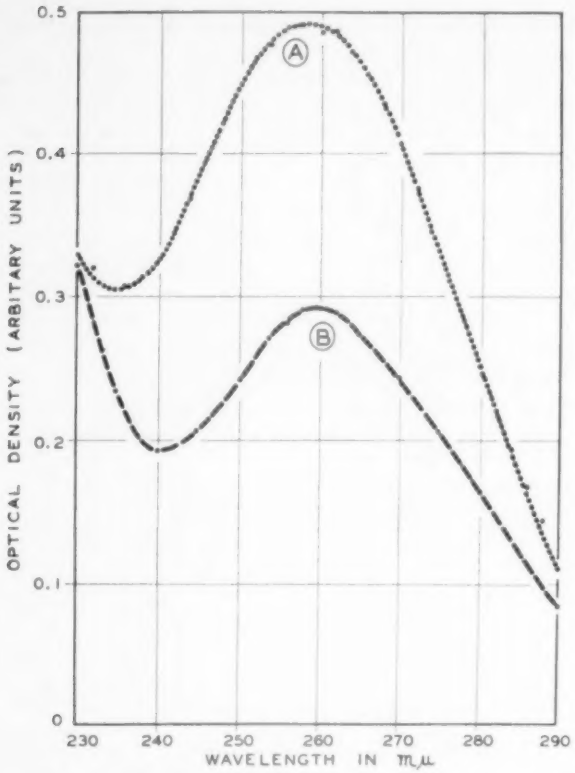


**Fig. 3.** Ultraviolet light absorption spectra of purified preparations of cucumber mosaic virus. A) preparation from stage 4 in Fig. 2; B) preparation from stage 8 in Fig. 2.

leaves after butanol treatment and ultracentrifugation. The virus preparations were highly opalescent and highly infectious. Similar preparations of healthy leaves were slightly opalescent, and, like the virus preparations, they absorbed strongly in ultraviolet light at 260 mμ. Attempts were made to separate this host material from the virus preparations by sedimentation in sucrose gradients, as described by Brakke (3).

One to two ml of these preparations were layered over a gradient ranging from 5 to 40% sucrose in *0.05M* buffer and centrifuged for varying periods at 25,000 rpm in the SW 25.1 swinging bucket rotor. A faint opalescent band was visible in the gradient containing the healthy preparation after centrifugation. This band coincided in distance with a white band in the gradient layered with the virus preparation. Since this white band was later shown to contain the virus, it was concluded that the virus and the host constituents in these preparations could not be separated by this method. Tests were made to remove these host constituents by precipitation with acid.

*Acid precipitation.*—A partially purified virus preparation was divided into 7 equal volumes. They were placed in an ice bath, and pH was lowered by drop-wise addition of 10% acetic acid with constant stirring. Different volumes were acidified to give a range of pH values of 6.5–4.0. The acidified solutions were centrifuged immediately for 20 minutes at 10,000 rpm. The supernatants were decanted, adjusted to pH 7.5 with 10% ammonium hydroxide, and, after being made up to equal volumes, assayed on cowpea. The pellets were resuspended for 2 hours in 0.5 ml of *0.05M* buffer, and clarified by low-speed centrifugation, and the clear supernatants were assayed on cowpea after being made up to a volume equal to that of the supernatants. The results (Table 2) demonstrated that the virus could be precipitated from solution at pH 5.0. A heavy white precipitate containing all the infectivity formed with acidification. Resuspension and slow-speed centrifugation of this precipitate gave a clear virus solution that absorbed strongly with ultraviolet light at 260 mμ. Similar acidified preparations from healthy plants gave no such absorption.

*Further treatment.*—The virus was further purified by ultracentrifugation. The virus preparations were centrifuged 2 hours at 30,000 rpm. The supernatant was discarded, and the clear glasslike pellet was resuspended for 2 hours in a small volume of either *0.05M* buffer or distilled water. Slow-speed centrifugation for 10 minutes at 5000 rpm produced a clear virus solution.

A summary of the purification procedure is described diagrammatically in Fig. 2.

*Ultraviolet absorption.*—The ultraviolet absorption

TABLE 3.—*A precipitin ring test between a partially purified CMV preparation and its antiserum*

| Dilution of antiserum | Dilution of virus preparation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | 1/128 | 1/256 | 1/512 | 1/1024 |
| 1/2 | ++++[a] | ++++ | ++++ | +++ | +++ | +++ | +++ | ++ | ++ | ++ |
| 1/4 | ++++ | ++++ | ++++ | +++ | +++ | +++ | +++ | ++ | ++ | ++ |
| 1/8 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | ++ | ++ | ++ |
| 1/16 | +++ | +++ | +++ | +++ | +++ | ++ | ++ | ++ | ++ | + |
| 1/32 | +++ | ++ | ++ | ++ | ++ | ++ | ++ | + | + | + |
| 1/64 | +++ | ++ | ++ | ++ | ++ | ++ | + | + | + | + |
| 1/128 | +++ | ++ | ++ | + | + | + | + | + | + | - |
| 1/256 | ++ | + | + | + | + | + | + | + | - | - |
| 1/512 | ++ | + | + | + | + | + | + | - | - | - |
| 1/1024 | ++ | + | + | + | - | - | - | - | - | - |

[a] The intensity of the precipitate is indicated by the number of + signs; - indicates no precipitate.

TABLE 4.—*The reaction in a tube test between CMV antiserum and partially purified preparations of CMV-infected and healthy tobacco leaves*

| Antigen dilutions | Antiserum dilutions | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 | 1/128 | 1/256 | 1/512 | 1/1024 |
| Healthy preparation 1/5 | +[a] | ± | − | − | − | − | − | − | − | − |
| Healthy preparation 1/10 | ± | − | − | − | − | − | − | − | − | − |
| CMV preparation 1/5 | ++++ | ++++ | ++++ | ++++ | ++++ | +++ | ++ | + | + | ± |
| CMV preparation 1/10 | ++++ | ++++ | ++++ | +++ | +++ | +++ | ++ | + | ± | − |

[a] The intensity of the precipitate is indicated by the number of + signs; − indicates no precipitate.

of CMV was measured with a Beckman DU spectrophotometer. The absorption spectra of 2 such preparations are shown in Fig. 3, curves A and B showing respective absorption of preparations at stages 4 and 8, as described in Fig. 2. Both preparations showed curves typical of nucleoproteins with maxima near 260 mμ and minima at 235–240 mμ. The absorption ratios at the 260- and 280-mμ wave lengths differed with preparations before and after acid precipitation, indicating that some of the material absorbing at 260 mμ was removed by this treatment.

ELECTRON MICROSCOPY AND ULTRACENTRIFUGAL ANALYSIS.—Purified preparations of CMV in distilled water were sprayed, or added as drops, on collodion-coated electron-microscope grids, and allowed to air-dry. As controls, grids were also treated with similar preparations from healthy tobacco leaves. The grids were shadowed with uranium and examined with an RCA-type EMU2 electron microscope. The electron micrographs of the partially purified virus preparation (stage 4, Fig. 2) showed numerous spherical particles (Fig. 4). They were not found in micrographs of the control preparations, and it was concluded that they were particles of CMV. They had a mean diameter of 40 mμ, in comparison with polystyrene latex particles, which served as internal size-standards. The electron micrographs of a virus preparation obtained at stage 8 in Fig. 2 showed the same characteristic particles.

The homogeneity of a purified CMV preparation was tested in the Spinco analytical ultracentrifuge. A single schlieren peak was observed during centrifugation, indicating that such preparations contained a single component. The sedimentation constant of this component was 101 S.

SEROLOGICAL TESTS.—*Ring tests.*—The serum from

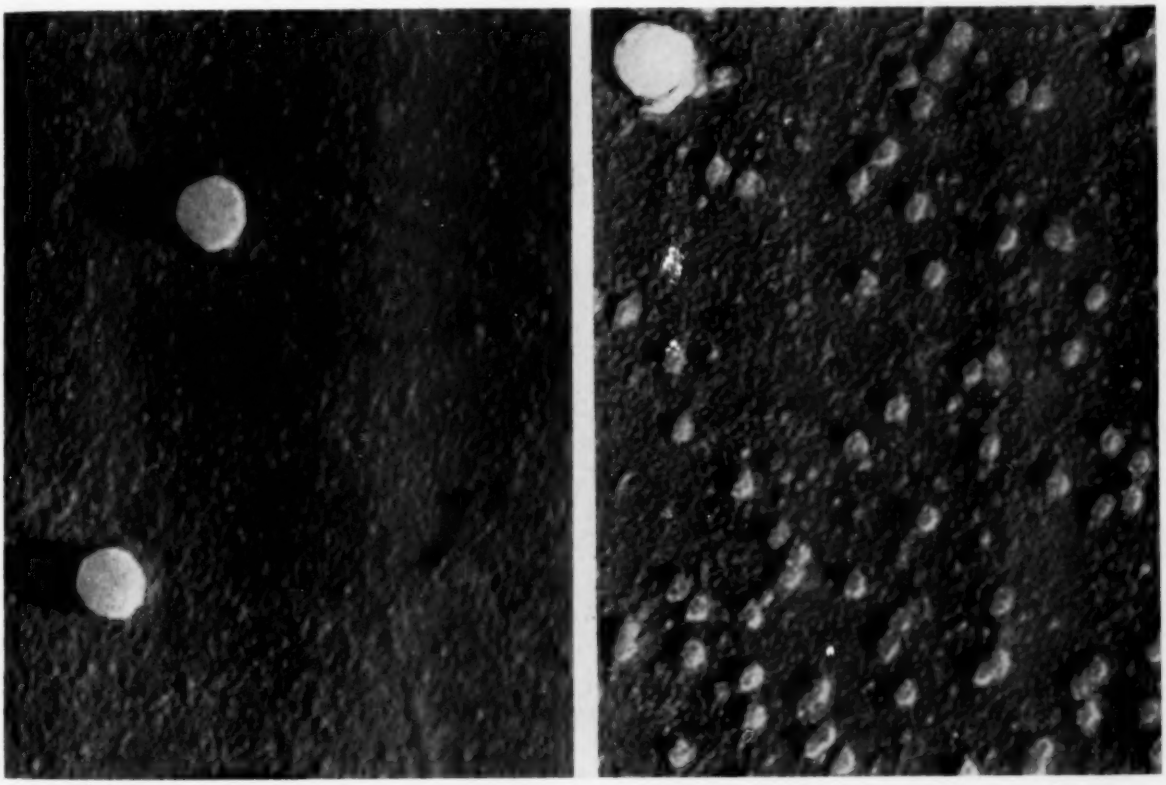

**Fig. 4.** Electron micrographs of partially purified preparations of CMV-infected **(right)** and healthy **(left)** tobacco leaves, (× 70,000). The large white spheres are polystyrene latex particles, 133 mμ in diameter.

TABLE 5.—*The reaction in a tube test between CMV antiserum and unpurified sap of CMV-infected and healthy tobacco leaves*

| Source of Sap | Antiserum dilutions | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1/1 | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | 1/64 |
| Healthy leaves | +++[a] | ++ | + | + | ± | − | − |
| Infected leaves | ++++ | ++++ | ++++ | +++ | ++ | + | ± |

[a] The intensity of the precipitate is indicated by the number of + signs; − indicates no precipitate.

rabbits injected with preparations of the partially purified CMV (stage 4, Fig. 2) gave a strong precipitin reaction when tested against similar preparations. A precipitate formed at the antiserum-antigen interface in 1–2 minutes at 25°C. With lower dilutions of virus, the heavy precipitate that formed usually settled to the bottom of the tube in an hour. No precipitate formed when similar tests were made with normal serum. Tests with partially purified preparations of healthy tobacco leaves gave a faintly visible precipitate against the undiluted antiserum, but not with antiserum diluted 1/5 or with normal serum. The serum, therefore, did not require absorption with healthy tobacco preparations when used at dilutions of 1/5 or more. When serial twofold dilutions of antiserum and partially purified CMV were used, a titer of 1/1024 was obtained (Table 3).

*Tube test.*—Partially purified preparations of CMV-infected and healthy tobacco leaves were used in tests to confirm the specificity of the antiserum. In one such test with a CMV preparation diluted 1/5, the antiserum titer was about 1/1024 (Table 4).

Tests were made with unpurified sap from CMV-infected and healthy tobacco leaves. The sap was centrifuged at slow speed to remove coarse cellular debris, and the undiluted supernatants were tested against serial twofold dilutions of normal serum and antiserum. No precipitation occurred when either sap was mixed with normal serum. The reaction between the saps and the antiserum, recorded 20 minutes after the beginning of the test, showed that the antiserum could be used for detecting CMV in unpurified sap (Table 5). After mixing for 1 hour, however, spontaneous precipitation occurred and no differences were visible between the titers of the two series.

DISCUSSION.—The ease with which different viruses can be purified varies considerably, and procedures that work well with some fail with others. Thus, some viruses can be frozen and thawed, heated to fairly high temperatures, and exposed to high concentrations of salt, ethanol, and various other organic solvents without loss of infectivity. These methods, singly or in combinations, have been used successfully in removing noninfective material from infected host extracts. Many other viruses, however, lose infectivity when similarly treated, and, in the present studies, these treatments caused either much or complete inactivation of CMV.

With several unstable viruses, less harsh methods of purification have been used, such as differential centrifugation and zonal separation in sucrose gradients, but such methods were unsuccessful with CMV. Thus, as previously mentioned, slow-speed centrifugation of CMV-infected host extracts caused the removal of the virus from the clarified supernatant. The supernatants of strongly buffered extracts, clarified with butanol and slow-speed centrifugation, however, were still highly infectious. This treatment may be bringing virus into solution, because homogenates treated with butanol, as shown in Table 2, appeared to contain more virus than untreated homogenates. It is possible, therefore, that butanol disrupts the association of virus and certain host components. The mechanism is not understood, but it may be that butanol acts in a way similar to that in the solubilization of various enzymes, as discussed by Morton (7). Whatever the explanation, however, the decisive step in the purification outlined for CMV is the clarification of extracts with butanol, for only thus was it possible to keep the virus in solution during slow-speed centrifugation.

Applicability to other viruses of the method of purification described is not known. Alfalfa mosaic virus, another aphid-transmitted virus, was originally purified by Ross (8), and later by Bancroft and Kaesberg (1). The latter workers used Steere's method (12), employing a two-phase butonal-chloroform mixture, but the relative importance of the two organic solvents was not discussed.

Electron micrographs of CMV obtained by previous workers with unpurified extracts showed the virus as a spherical particle ranging in size from 91–120 mμ (5) to 35 mμ (11). Electron micrographs of purified preparations of the virus in the present study have confirmed these earlier observations on the shape of the virus, but demonstrated that the virus is a uniform-sized particle with a mean diameter of 40 mμ.

Further serological work is in progress, for it is hoped that by such means the relationships of CMV strains can be determined.

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# FURTHER STUDIES OF PHYSIOLOGIC RACES IN UROCYSTIS TRITICI [1]

By A. G. Johnson [2]

SUMMARY

Seed of various wheat varieties were inoculated with flag smut *(Urocystis tritici)* from Chili, Australia, South Africa, Japan, India, and China, and grown in a greenhouse in comparison with U.S. races 1 and 2. Results indicate the fungus from Chili is race 2; that from Australia, though similar to U.S. race 2, is considered a separate biotype (designated race 2A); that from South Africa, differing somewhat from U.S. race 2 and race 2A, is considered a separate biotype (designated race 2B); that from Japan, distinctly different from all the above, is designated race 3; that from India, also different from all the above, is designated race 4; Chinese races C1 and C2 are fairly similar to U.S. 1, but the other Chinese races differ in various respects from all the other collections.

In a previous article,[3] 2 physiologic races of *Urocystis tritici* Koern. from collections in the United States were described as races 1 and 2. These were distinguished primarily by the differential reactions of Oro × Federation-38, Oro × Federation-40, and Oro × Federation-1. Under similar greenhouse conditions at Pullman, Washington, Arlington Farm, Virginia, and Beltsville, Maryland, in 1941 and 1942, the first two showed high resistance to race 1 and rather high susceptibility to race 2, and the third variety was highly susceptible to both races.

In 1936, Yu, Hwang, and Tsiang,[4] and in 1945, Yu, Wang, and Fang,[5] reported studies on rather extensive collections of *U. tritici* in China and described 12 races of this fungus.

In 1951, Hafiz,[6] reported studies of 11 collections of the same species of smut fungus from 6 different countries: the United States, Italy, Australia, China, Cyprus, and Pakistan. These collections were compared with U.S. races 1 and 2 on a number of wheat varieties, including Oro × Federation-38. His collections fell clearly into 4 groups: group 1 was U.S. race 1, from the United States only; group 2, from Italy and Australia, agreed with U.S. race 2; group 3, from China and Cyprus, was undetermined since no infection was obtained on the wheats used in the experiments; group 4, 2 collections from Pakistan, distinctly different from the others and therefore considered a separate race, produced rather heavy infection on the wheat variety Punjab T9, and no infection on other wheats tested.

MATERIAL AND METHODS. — Following the earlier work,[3] additional studies of physiologic specialization were continued by the writer at the Plant Industry Station, Beltsville, until his retirement, in 1949. The methods used were essentially the same as previously described.[3]

To compare the pathogenicity of the flag smut fungus from other countries with that of our races 1 and 2 under similar conditions, collections were obtained through the generous cooperation of scientists and their associates in Chile, Australia, China, South Africa, Japan, India, Egypt, and Pakistan. These collections and wheat samples were received through the then Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, which approved their use under greenhouse conditions carefully guarded so as to avoid escape of the pathogens. These smut collections and wheat samples were received from: Sergio Tartakowsky, Chile; Dr. W. L. Waterhouse, Australia; Dr. Len Verwoerd, South Africa; Dr. S. C. Salmon, Japan; Dr. B. B. Mundkur, India; Dr. T. L. Tai, China; Dr. A. F. El-Helaly, Egypt; and Dr.

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TABLE 1.—*Average percentages of smutted plants in 33 wheat varieties inoculated with 20 collections of chlamydospores of Urocystis tritici from different countries*[a]

| Source and Variety | | Percentage of smutted plants following inoculation with collection numbers: | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | C1[b] | C2 | C3 | C4 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| United States | C.I.no. | | | | | | | | | | | | | | | | | | | | |
| Baart | 1697 | 18 | 20 | 22 | 36 | 55 | 61 | 32 | 5 | 9 | 5 | 2 | 31 | 15 | 18 | 30 | 12 | 21 | 15 | 15 | 3 |
| Baart—46 | | 2 | 3 | — | 9 | 16 | 4 | 13 | 2 | 2 | 2 | 0 | 16 | 2 | 3 | 2 | 6 | 2 | 11 | 10 | 0 |
| Elgin | 11755 | 0 | 0 | 0 | 4 | — | — | 4 | — | — | — | — | 2 | 0 | — | 0 | 0 | 0 | 3 | 0 | 0 |
| Federation | 4734 | 44 | 44 | 31 | 52 | 84 | 84 | 58 | 20 | 13 | 23 | 16 | 31 | 21 | 23 | 31 | 26 | 28 | 27 | 8 | 7 |
| Golden | 10063 | 0 | 0 | 0 | 0 | — | 0 | 0 | — | — | 0 | — | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ghurka—616 | | 0 | 7 | 12 | 18 | 11 | 12 | 1 | 0 | 0 | 0 | 0 | 4 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Kanred x Hard Federation | 10092 | 35 | 28 | 27 | 70 | 92 | 77 | 56 | 32 | 5 | — | — | 49 | 22 | 85 | 23 | 56 | 92 | 38 | — | — |
| Orfed | 11913 | tr[c] | 1 | 2 | 4 | 11 | 4 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 6 | 0 | 0 | tr | 0 | 0 |
| Oro x Federation—1 | 11914 | 38 | 38 | 41 | 73 | 95 | 93 | 73 | 41 | 0 | 60 | 70 | 50 | 42 | 77 | 53 | 50 | 80 | 49 | 8 | 60 |
| Oro x Federation—38 | | tr | 37 | 40 | 54 | 74 | 78 | 52 | 0 | 10 | 0 | 0 | 10 | 2 | 12 | 31 | 0 | 0 | 32 | 0 | tr |
| Oro x Federation—40 | | 0 | 71 | 50 | 69 | 75 | 61 | 87 | 0 | 0 | — | — | 6 | 2 | 8 | 78 | 0 | 0 | 52 | — | — |
| Rex | 11689 | 0 | 0 | 0 | 12 | — | — | 16 | — | — | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| China | P.I.no. | | | | | | | | | | | | | | | | | | | | |
| Ngochen | 149805 | tr | 17 | 16 | 27 | 29 | 31 | 12 | 10 | 3 | 0 | 0 | 20 | 19 | 19 | 10 | 11 | 18 | 27 | 2 | 2 |
| Grassland | 149806 | 0 | tr | tr | 2 | 2 | 4 | 0 | 2 | 10 | 0 | 0 | 7 | 10 | 24 | 1 | 0 | 2 | 30 | 0 | 0 |
| Tsing Hua 677 | 149807 | 0 | tr | 0 | 0 | tr | 1 | 0 | tr | 0 | 0 | 0 | 5 | 2 | 4 | 0 | 2 | 2 | 1 | 0 | 0 |
| N. 716 | 149808 | 0 | 7 | 12 | 9 | 13 | 19 | 2 | 0 | 3 | 0 | 0 | 1 | 2 | 5 | 6 | 0 | 0 | 27 | 0 | 0 |
| T.H. 1932 | 149809 | tr | 1 | 2 | 0 | tr | tr | tr | 0 | 2 | 0 | 0 | 2 | 0 | 5 | tr | 0 | 5 | 1 | 0 | 0 |
| T.H. 3929 | 149810 | 29 | 48 | 68 | 90 | 92 | 100 | 55 | 94 | 5 | 29 | 38 | 51 | 69 | 48 | 30 | 66 | 43 | 52 | 33 | 31 |
| T.H. 559 | 149812 | 0 | 0 | 0 | 0 | — | 0 | 0 | — | — | — | — | 0 | 0 | — | 0 | 0 | — | 0 | — | 0 |
| Africa | | | | | | | | | | | | | | | | | | | | | |
| Spring Eearly | 166410 | 24 | 33 | 28 | 39 | 55 | 48 | 41 | 16 | 0 | — | — | 32 | 34 | 37 | 31 | 16 | 50 | 24 | — | — |
| India | | | | | | | | | | | | | | | | | | | | | |
| N.P. 101 | 159916 | 0 | 0 | — | — | 2 | 2 | 0 | 2 | 91 | — | — | — | — | — | — | — | — | — | — | — |
| V—184 | 159919 | 0 | 5 | — | — | 7 | 0 | 1 | 0 | 40 | — | — | — | — | — | — | — | — | — | — | — |
| N.P.4 | 159913 | 0 | 0 | — | — | 11 | 2 | 0 | 2 | 0 | — | — | — | — | — | — | — | — | — | — | — |
| N.P.12 | 159914 | 0 | 13 | — | — | 31 | 19 | 10 | 21 | 24 | — | — | — | — | — | — | — | — | — | — | — |
| N.P.80-5 | 159915 | 0 | 2 | — | — | 26 | 19 | 3 | 0 | 0 | — | — | — | — | — | — | — | — | — | — | — |
| N.P.165 | 159917 | 0 | 29 | — | — | 35 | 63 | 59 | 52 | 28 | — | — | — | — | — | — | — | — | — | — | — |
| Punjab 8-A | 159918 | 0 | 9 | — | — | 20 | 8 | 4 | 35 | 27 | — | — | — | — | — | — | — | — | — | — | — |
| Egypt | | | | | | | | | | | | | | | | | | | | | |
| Baladi "Bouhi" | 171406 | 0 | 0 | — | — | 0 | 0 | 0 | 0 | 0 | — | — | — | — | — | — | — | — | — | — | — |
| Baladi 116 | 171407 | 0 | 0 | — | — | 0 | 0 | 0 | 0 | 0 | — | — | — | — | — | — | — | — | — | — | — |
| Baladi, Dakar | 171408 | 0 | 0 | — | — | 0 | 0 | 0 | 0 | 0 | — | — | — | — | — | — | — | — | — | — | — |
| Hindi "D" | 171409 | 4 | 28 | — | — | 23 | 15 | 45 | 0 | 76 | — | — | — | — | — | — | — | — | — | — | — |
| Hindi 62 (Dahabi) | 171410 | 4 | 60 | — | — | 71 | 63 | 73 | 12 | 81 | — | — | — | — | — | — | — | — | — | — | — |
| Kazouria "Taliani" | 171411 | 2 | 2 | — | — | 2 | 0 | 4 | 0 | 13 | — | — | — | — | — | — | — | — | — | — | — |

[a] Nos. 1 and 2 (U.S. races 1 and 2), United States; No. 3, Chile; Nos. 4–6, Australia; No. 7, South Africa; No. 8, Japan; No. 9, India.
[b] Chinese races 1–4 and 6–12 are here designated C1–C4 and C6–C12.
[c] tr = trace.

Abdul Hafiz, Pakistan.

During 1945–1949 the above collections, together with our races 1 and 2, were tested for 1–5 years, as spore material and greenhouse space were available, on 33 varieties of wheat: all common wheat, *Triticum aestivum* L. (*T. vulgare* Vill.), except Chinese T.H. 559, which was a poulard, *T. turgidum* L.

In practically all tests, dry flag smut spores were applied abundantly to wheat seed in tight coin envelopes, which were then tightly closed and shaken to distribute the spores on the kernels. In a few cases in which spore material was especially meager, the water-suspension method of inoculating the kernels was used, with comparable results. Painstaking precautions were taken throughout to avoid mixing the strains of smut spores.

Plantings were made in the greenhouse, about 2–2½ in. deep, in moderately moist loam soil, about 100 kernels per 58-in. row or 75 kernels per 34-in. row. Temperatures were kept at 15–20° C throughout the emergence period.

At or near the maturity of normal plants, the percentage of completely and partly smutted plants was recorded. The percentages given are averages of the percentages obtained in different years. The percentage of smutted plants differed somewhat from year to year in any one wheat variety inoculated with any one smut collection, yet the relative susceptibility or resistance remained fairly constant. In some plants of some varieties, smut became evident only on the neck or head of the plants, and taking final data was necessarily delayed to be sure that all smutted plants were detected.

RESULTS AND DISCUSSION.—The average percentages of smutted plants in the wheat varieties included in the experiments are given in Table 1. With most wheat varieties the smut percentages shown are averages of results obtained during 3–5 years, 1 crop per year.

TABLE 2.—*Ratings*[a] *of wheat varieties that showed differential reactions to flag smut collections 1–9, C1–C4, and C6–C12 in Table 1.*

| Variety | Collection Numbers | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1[b] | 2[c] | 3[c] | 4–6[d] | 7[e] | 8[f] | 9[g] | C1[h] | C2 | C3 | C4 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| Baart | I+ | I+ | I+ | S | S | R | I– | R | R | S | I | I | S | I | S– | I | I | R |
| Ghurka 616 | R | I– | I | I | R– | R– | R– | R– | R– | R | R | R– | R– | R– | R– | R– | R– | R– |
| Kanred x Hard Federation | S | S | S | S+ | S | S | R+ | – | – | S | S | S+ | S | S | S+ | S | – | – |
| Oro x Federation—1 | S | S | S | S+ | S+ | S | R– | S+ | S+ | S | S | S+ | S | S | S+ | S | I– | S+ |
| Oro x Federation—38 | R– | S | S | S+ | S | R– | I | R– | R– | I | R | I | S | R– | R– | S | R– | R– |
| Oro x Federation—40 | R– | S+ | S | S+ | S+ | R– | R– | – | – | I– | R | I | S+ | R– | R– | S | – | – |
| Rex | R– | R– | R– | I | I | – | – | R– | R– | R– | R– | R– | R– | R– | R– | R | R | R– |
| Ngochen | R– | I | I | S | I | I | R | R– | R– | I+ | I+ | I+ | I | I | I | S | R | R |
| Grassland | R– | R– | R– | R | R | R | I | R– | R– | I– | I | S | R | R– | R | S | R– | R– |
| N. 716 | R– | I– | I | I | R | R– | R | R– | R– | R | R | R+ | R | R– | R– | S | R– | R– |
| T.H. 3929 | S | S | S+ | S+ | S | S+ | R+ | S | S | S | S+ | S | S | S+ | S | S | S | S |
| Spring Early | I+ | S | S | S | S | I+ | R– | – | – | S | S | S | S | I+ | S | S | – | – |
| N.P.—101 | R– | R– | – | R | R– | R | S+ | – | – | – | – | – | – | – | – | – | – | – |
| V—184 | R– | R+ | – | R | R– | R– | S | – | – | – | – | – | – | – | – | – | – | – |
| N.P.—4 | R– | R– | – | I– | R– | R | R– | – | – | – | – | – | – | – | – | – | – | – |
| N.P.—12 | R– | I | – | S | I | S– | S– | – | – | – | – | – | – | – | – | – | – | – |
| N.P.—80-5 | R– | R | – | S | R | R– | R– | – | – | – | – | – | – | – | – | – | – | – |
| N.P.—165 | R– | S | – | S | S | S | S | – | – | – | – | – | – | – | – | – | – | – |
| Punjab 8A | R– | I | – | I | R | S | S | – | – | – | – | – | – | – | – | – | – | – |
| Hindi "D" | R | S | – | I | S | R– | S+ | – | – | – | – | – | – | – | – | – | – | – |
| Hindi 62 | R | S+ | – | S+ | S+ | I | S+ | – | – | – | – | – | – | – | – | – | – | – |

[a] R = resistant, 0–5% smutted; I = intermediate, 6–20% smutted; S = susceptible, 21–100% smutted (– after letters indicates lower percentage range; +, higher range).
[b] U.S. race 1; [c] U.S. race 2; [d] biotype 2A; [e] biotype 2B; [f] new race 3; [g] new race 4; [h] C1–4, C6–12 are Chinese races 1–4, 6–12.

The duration of the tests with the various flag smut collections was as follows: Nos. 1[7] (U.S. race 1) and 2 (U.S. race 2), 5 years; No. 3 from Chile, 3 years; Nos. 4–6 from Australia, 3 years; No. 7 from South Africa, 4 years; No. 8 from Japan, 3 years; and No. 9 from India, 3 years; C1–C4 and C6–C12 from China, 2–4 years. The Indian and Egyptian wheats were included in only 1 year (1949). Two of the collections from Egypt were included in 1949, but as only very low percentages of smutted plants were obtained (highest only 2%), these results are not considered significant and therefore are not included. The collection from Pakistan was received in 1949, too late to be included in these tests. Throughout the tests, the U.S. races 1 and 2 (Nos. 1 and 2) remained distinct in degrees of pathogenicity on Oro × Federation-38 and Oro × Federation-40, as previously reported.[3] In addition, these races also showed differences in pathogenicity on the Chinese variety Ngochen, the Indian varieties N.P.-12 and N.P.-165, and Egyptian, Hindi "D" and Hindi 62, which were susceptible to U.S. race 2 and resistant to Race 1. In practically all of the wheats included, race 2 showed the greater virulence.

The smut from Chile, No. 3, fell clearly with U.S. race 2, the smut percentages throughout the series being almost identical.

It is evident from Table 1 that all three smut collections from Australia (Nos. 4, 5, and 6) were very similar in degree of pathogenicity throughout the series. Their pathogenicity is very similar to that of U.S. race 2, as reported by Hafiz,[6] but their virulence on most varieties throughout the series was distinctly greater than that of U.S. race 2. Furthermore, No. 4 produced 12% of smutted plants on Rex though no smutted plants were produced in this variety by U.S. race 2 in the years it was used on it. Unfortunately, Nos. 5 and 6 were not used on this variety. It seems, therefore, that the Australian smut is similar to U.S. race 2, but has proved distinctly more virulent and, in addition, attacked the variety Rex somewhat, though U.S. race 2 did not. It is therefore here considered a distinct biotype, and designated race 2A.

The smut from South Africa (No. 7) also was similar to U.S. race 2 in degree of pathogenicity, but, like the Australian collections, it showed distinctly greater virulence on most varieties and was able to attack Rex, in which it produced 16% smutted plants. This smut differed from the Australian collections, however, in being distinctly less virulent on Ghurka-616, Chinese N-716, and Indian: N.P.-4 and N.P.-80-5. It therefore seems that this South African smut differs sufficiently from the Australian collections, biotype 2A, to be designated a separate biotype, 2B.

The smut from Japan (No. 8) was similar to U.S. race 1 in that it infected neither Oro × Federation-38 nor Oro × Federation-40. It differed distinctly, however, in producing 21, 52, and 35% smutted plants on Indian wheats respectively, N.P.-12, N.P.-165, and Punjab 8-A—whereas U.S. race 1 produced no smutted plants at all in any of these wheats. The Japanese smut produced only 5% smutted plants in Baart, whereas practically all of the other smut collections produced much higher percentages on this variety. The Japanese smut (No. 8) is therefore considered a distinct race, and designated race 3.

[7] Collection numbers as used in Tables 1 and 2.

The smut from India (No. 9) failed to infect Oro × Federation-40, although it infected Oro × Federation-38 slightly (10%). Curiously enough, it failed to infect, or produced only very low percentages of smutted plants in, Kanred × Hard Federation, Oro × Federation-1, Chinese T.H. 3929, and African Spring Early, all of which were susceptible or highly susceptible to all other smuts tested. No. 9 produced 91 and 40% smutted plants, respectively, on the Indian N.P.-101 and V-184 wheats, known to be susceptible in India, though these two wheats were intermediate or, in most cases, highly resistant to all the other smuts used on them. Thus it is clear that the Indian smut is a distinct race, and is here designated race 4. This may be the same race as that described by Hafiz,[6] from Pakistan, but this was not determined.

In general the Chinese races[5] were similar to most of the other races in that the most susceptible varieties (Baart, Federation, Kanred × Federation, Oro × Federation-1, Chinese T.H. 3929, and African Spring Early) were in most cases attacked rather severely, and the resistant varieties (Elgin, Golden, Rex, and Chinese T.H. 559) were attacked sparingly or not at all. In other respects the Chinese races differed from most of the other races used; and in most cases they also differed from each other.

The pathogenicity of the Chinese races C1 and C2 was fairly similar to that of U.S. race 1, but U.S. 1 attacked Baart distinctly more severely than did either C1 or C2.

Chinese C3 was somewhat similar to U.S. 2, except that C3 attacked Baart and Baart-46 more severely, and Oro × Federation-38 and Oro × Federation-40 distinctly less severely.

Chinese C4 was somewhat similar to U.S. 1 except that it attacked Oro × Federation-38 and Oro × Federation-40 very slightly, and Grassland to an intermediate degree. These varieties were highly resistant to U.S. 1.

Chinese C6 was similar to C4, except that C6 was distinctly the more virulent on Kanred × Hard Federation, Oro × Federation-1, Oro × Federation-38, Oro × Federation-40, and Grassland. Both C4 and C6 differed from U.S. 1 and 2 in attacking Grassland.

Chinese C7 agreed with U.S. 2 fairly well on the wheats on which it was used.

Chinese C8 was fairly similar to U.S. 1, except that it attacked the Chinese wheat Ngochen distinctly, whereas U.S. 1 attacked that variety scarcely at all.

Chinese C9 was similar to C8, but distinctly the more virulent on Kanred × Hard Federation and Spring Early. Both C8 and C9 were similar to U.S. 1 in not attacking Oro × Federation-38 or Oro × Federation-40.

Chinese C10 was somewhat similar to U.S. 2, except that it attacked Grassland rather severely (30%) whereas U.S. 2 attacked it scarcely at all. Also, C10 attacked Chinese N716 rather severely (27%) whereas U.S. 2 attacked this variety only slightly (7%).

Chinese C11 was similar to U.S. 1 in not attacking Oro × Federation-38, but C11 was distinctly less virulent on Federation and Oro × Federation-1.

Chinese C12, originally collected on a poulard wheat in China, also was somewhat similar to U.S. 1, and, like C11, was less virulent on Federation than U.S. 1, but was distinctly more virulent on Oro × Federation-1 and Chinese T.H. 3929 than either C11 or U.S. 1. Though C12 has been characterized as attacking Chinese T.H. 559, a poulard wheat, in China, it did not attack this wheat in the experiments here reported. This of course means that the environment where the experiments were conducted in the field in China[5] was more favorable for infection of that wheat than was the environment in the greenhouse where the present experiments were conducted.

Thus, 7 of the Chinese races used (C1, 2, 4, 8, 9, 11, and 12) were somewhat similar to U.S. race 1, but differed in various respects. One of the Chinese races (C7) agreed fairly well with U.S. race 2, while two others (C3 and C10) were somewhat similar to U.S. race 2, though differing in certain particulars and differing from each other. One of the Chinese races (C6) was distinctly different from both U.S. races 1 and 2.

The relative flag smut reactions of the wheats that have shown differential effects to the various smut collections (Nos. 1–9, C1–4 and C6–12, in Table 1) are given in Table 2. In general, the smut races are segregated by smut ratings at least as different as "R–" and "S" on one or more wheat varieties or strains, and biotypes by lesser, yet significant, differences in such smut ratings.

It should be noted that in the studies here reported, as shown in Table 1, Golden and the three Egyptian wheats, Baladi Bouhi, Baladi 116, Baladi Dakar, and Chinese Tsing Hua 677 all remained free from smut. In the field in the State of Washington, however, Golden has been known to have up to 20% of plants smutted by U.S. race 2. This of course indicates that results from tests under greenhouse conditions may not always be directly applicable to conditions in the field. This was also indicated above for Chinese race C12 and T.H. 559. That is, under favorable conditions in the field, flag smut percentages may be even higher than usually occur in the greenhouse. On the other hand, apparently the reverse may also occur. For example, Dr. C. S. Holton, in correspondence, reported that, in the State of Washington, the wheat variety Rex is usually susceptible to race 2 in the greenhouse but consistently resistant in the field.

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[5] For convenience of reference, the Chinese races are designated "C1, C2, etc.," in Tables 1 and 2, for the Chinese races 1, 2, etc. The Chinese races are not otherwise renumbered.

# MEASURING PATHOGENESIS BY THE AMOUNT OF TOXIC SUBSTANCE PRODUCED IN ALFALFA BY A SNOW MOLD FUNGUS [1]

J. B. Lebeau, [2] M. W. Cormack, [3] and J. E. Moffatt [2]

SUMMARY

When the development of winter crown rot was traced by making HCN determinations on the crown buds of inoculated alfalfa plants at 2-week intervals, the pathogen proved active beneath the snow in the winter as well as in the spring and fall. Mycelium was not present in the crown bud tissues until positive tests were obtained for HCN. The period Sept.–Nov. was shown to be critical in the disease cycle because only inoculations made before mid-November incited the disease.

It has been demonstrated that the winter crown rot pathogen (an unidentified low-temperature basidiomycete) synthesizes cyanide, HCN, which is absorbed by the crown tissues of the affected host plants (2). The present investigation was undertaken to learn more about the seasonal development of the disease and to determine whether HCN is absorbed by the plant before or after invasion of host tissues by the fungus.

HOST-PARASITE RELATIONSHIP.—Analyses of infected alfalfa plants from the field for the past 5 years show that every diseased plant contained HCN, in concentrations proportional to the damage in the host. This was evidence that HCN determinations can be used to measure the stage or severity of the disease.

A large block of first-year plants of *Medicago sativa* L. were inoculated in the field at Edmonton early in October by the method described by Cormack (1). A similar block of check plants were left uninoculated. Random samples of about 50 plants were taken from each block on the first and fifteenth of every month throughout the winter. The crown buds were immediately removed from the plants, and HCN determinations were made on half of the buds. The remaining half were fixed in formic acetic acid in preparation for sectioning. Techniques for embedding, sectioning, staining, and HCN determinations on the crown-bud tissues were essentially those outlined by Lebeau and Dickson (3). About 800 crown buds were analyzed for HCN content, and a like number were sectioned and examined histologically.

The development of snow mold of alfalfa was traced by this method during the winter of 1956–57 (Fig. 1). Sampling began on November 1, but no positive tests for HCN were obtained until December 15. The data show that the pathogen did not grow exclusively in the spring and fall, when temperatures approached the optimum for mycelial development, but that it was also active in January, February, and March. No HCN or evidence of the low-temperature basidiomycete was detected in the buds of uninoculated plants.

Cyanide concentration in inoculated crown buds dropped from 500 ppm in mid-March to 0 ppm at the end of April, when air temperatures rose well above the freezing point.

Sections of crown buds taken on each sampling date from December to April were critically examined for any evidence of mycelial invasion. In collections made in midwinter, masses of mycelium of the low-temperature basidiomycete were frequently observed in close attachment to the outer portions of the crown buds. This was particularly evident in the collections made on January 15 and February 1 (Fig. 2-A).

Mycelium was also observed between the outer scales and near the base and tip of rudimentary buds (Fig. 2-B). Occasionally the mycelium was massed into compact stromata similar to those sometimes produced in culture. These stromata were also observed in the form of infection pads, which initiated penetration through the tissues (Fig. 2-CD). In January and February the mycelium was confined in the tissues of the outer scales and leaf rudiments. In one section, however, the mycelium is shown massed at the tip of a bud rudiment (Fig. 2-E) with hypha extending into the scales surrounding the growing point. No invasion of the growing point was observed until March, when there was extensive mycelial invasion and disintegration of the buds (Fig. 2-F).

SEASONAL DEVELOPMENT.—To learn more about activity of the pathogen in the fall in relation to the severity of the disease, inoculations were made on

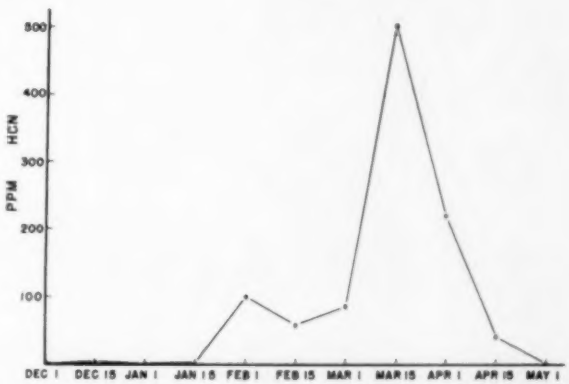


**Fig. 1.** Concentration of HCN recovered at different periods from crown buds of *Medicago sativa* inoculated with the low-temperature basidiomycete on October 8, 1957.

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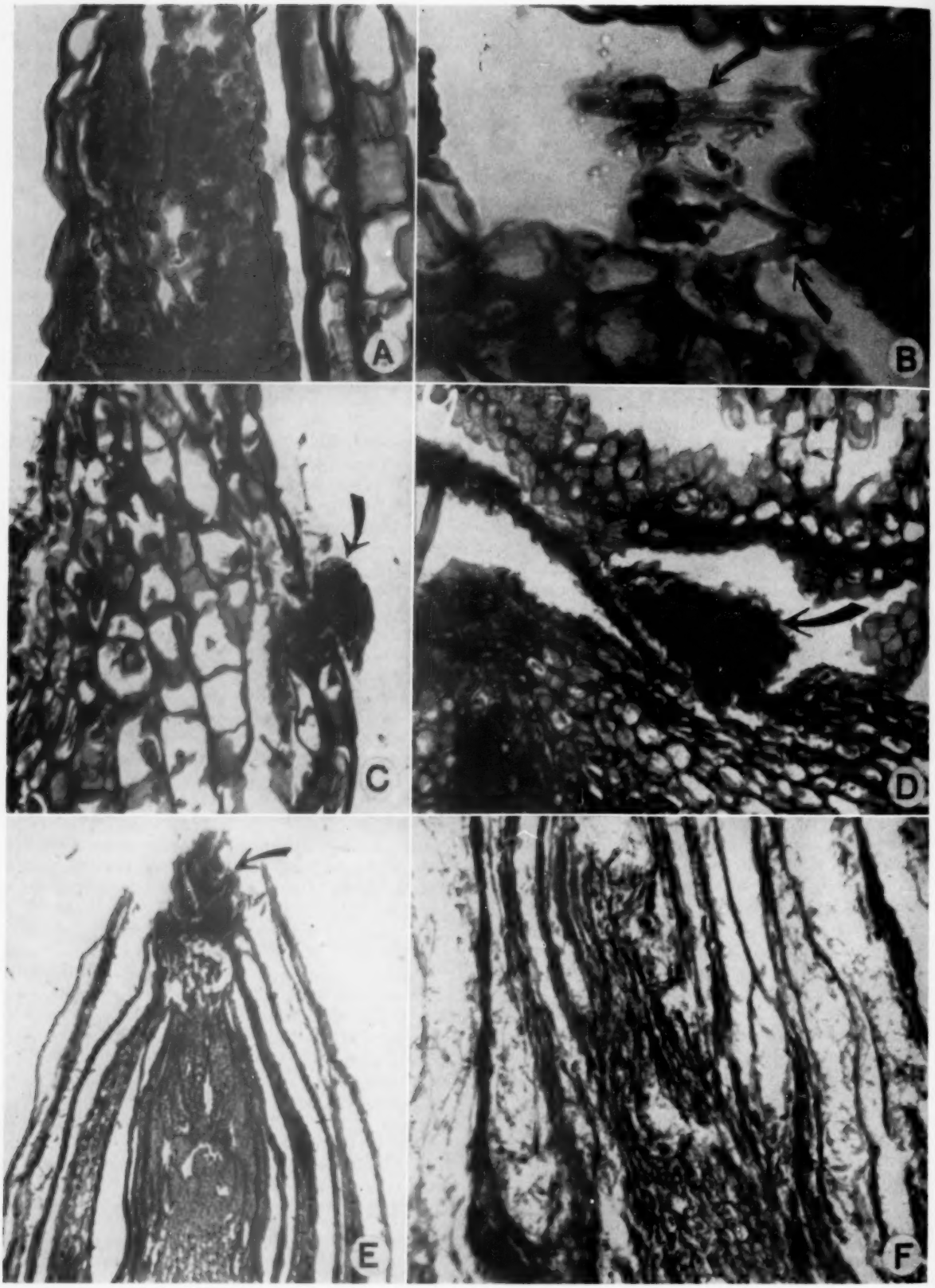
A
B
C
D
E
F

TABLE 1.—*Survival of Medicago sativa and M. falcata after inoculation with the winter crown rot basidiomycete on different dates*

| Date inoculated | Average survival (%) | |
|---|---|---|
| | *M. sativa* | *M. falcata* |
| Sep. 18, 1953 | 0.0 | |
| Sep. 22, 1954 | 22.0 | |
| Sep. 19, 1955 | 64.0 | 100.0 |
| Av. | 28.7 | |
| Oct. 1, 1953 | 0.0 | |
| Oct. 1, 1954 | 16.0 | |
| Oct. 4, 1955 | 12.0 | 78.0 |
| Av. | 9.3 | |
| Oct. 15, 1953 | 32.0 | |
| Oct. 15, 1954 | 78.0 | |
| Oct. 17, 1955 | 12.5 | 89.0 |
| Av. | 40.8 | |
| Nov. 1, 1953 | 67.0 | |
| Nov. 1, 1954 | 81.0 | |
| Nov. 2, 1955 | 30.0 | 90.0 |
| Av. | 59.3 | |
| Nov. 15, 1953 | 96.0 | |
| Nov. 15, 1954 | 85.0 | |
| Nov. 18, 1955 | 95.0 | 100.0 |
| Av. | 92.0 | |
| Check 1953 | 94.6 | |
| Check 1954 | 81.0 | |
| Check 1955 | 93.0 | 100.0 |
| Av. | 89.5 | |

first-year plants of *M. sativa* in 1953, 1954, and 1955. In 1955 a duplicate series of *M. falcata* was inoculated on the same dates. The plants were seeded in the spring in 12-ft. rows 1 ft. apart. Eight rows, chosen at random, were inoculated at about 15-day intervals from mid-September to mid-November, and 8 rows were left uninoculated in each test for controls. The plants were rated for survival in early June of the following spring. As shown in Table 1, average infection was greatest on plants inoculated near October 1, and no development of the disease was evident in any year in plants inoculated toward the middle of November. *M. falcata* showed the same response as *M. sativa* to different dates of inoculation, although it exhibited much greater resistance to the disease.

DISCUSSION.—Snow mold is generally considered to be a disease that develops early in the spring. No doubt, this popular conception is based on the luxuriant growth of the fungus that is evident on the ground surface in that season. The low-temperature basidiomycete, however, is probably harmless at that time, because the host does not absorb and retain HCN synthesized by the fungus when temperatures are much above freezing. This probably accounts for the failure to obtain artificial infection at temperatures approaching the optimum for growth of the pathogen (3). It does not imply, however, that no damage occurs in those springs when snow cover remains for a long period, preventing rapid dissipation of the HCN. Therefore, in low areas and other locations that accumulate snow, it is often advisable to remove it in the spring to reduce damage caused by the snow mold pathogen.

The results from the inoculation experiments (Table 1) indicate that the period between September and November is critical for development of the disease, regardless of such seasonal variations as temperature and snow cover. Failure to obtain infection when plants were inoculated on November 15 shows that the fungus must become established in the fall in order to incite the disease. This suggests that October is the logical month for the use of control measures in Western Canada, because chemicals or cultural practices that would hinder the development of the pathogen in this period should control the disease.

It was difficult to prove by chemical analysis and histological sections made from field samples that the plant tissues had absorbed cyanide previous to invasion by the fungus. However, there was no evidence of the low-temperature basidiomycete in the tissues prior to positive tests for HCN. Mass invasion and disorganization of the growing point of the buds were not observed until March 4, 2½ months after the first positive test for HCN in the tissues.

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**Fig. 2.** Different stages of infection of alfalfa crown buds by the low-temperature basidiomycete. **A)** Mycelium in close attachment to outer bud scales (390X), Feb. 1. **B)** Typical clamp connection of the fungus shown between the outer bud scales (510X), Feb. 1. **CD)** Stromata of the fungus in the form of infection pads (180X and 125X, respectively), March 1. **E)** Mycelium invading the apex of the crown bud (37X), Feb. 1. **F)** Mass invasion of the crown bud by the pathogen (125X), March 4.

# THE PATHOLOGY OF PHYTOPHTHORA LATERALIS ON NATIVE CHAMAECYPARIS LAWSONIANA [1]

Edward J. Trione [2]

SUMMARY

*Phytophthora lateralis* is the cause of a serious epiphytotic now spreading through the native range of *Chamaecyparis lawsoniana*. Sporangia, zoospores, chlamydospores and oospores are produced in the natural life cycle of this fungus. Seasonal development of aerial and soil inoculum is similar and parallel. The pathogen lies dormant throughout the warm, dry summer, develops very slowly in the fall and winter but increases rapidly in the spring. Resistant chlamydospores and oospores of *P. lateralis* are produced in infected foliar tissue. These spores presumably function as an important oversummering stage of the pathogen and apparently germinate only after an extended period of moderately cold weather. *P. lateralis* is well adapted to spread throughout the native range of *Chamaecyparis lawsoniana*. With no suitable control available the entire range of Port Orford cedar is seriously threatened by this pathogen.

INTRODUCTION.—In 1952, the serious root disease of *Chamaecyparis* caused by *Phytophthora lateralis* Tucker & J. Milbrath, was discovered in a small area in the native range of *Chamaecyparis lawsoniana* (A. Murr.) Parl. (4, 5). Previous work (2, 3, 5, 6, 9) on ornamental plantings of *Chamaecyparis*, dating back to 1937, established the extreme susceptibility of this tree to the pathogen. The moderate, moist, marine climate throughout much of the *Chamaecyparis* range is well suited to the growth of this pathogen (7). By 1954, the pathogen was widely distributed through the range of susceptible *Chamaecyparis* (4), and with no practical control available, a serious epiphytotic appeared imminent.

Shortly after this study began, in the summer of 1954, aboveground infections of *Chamaecyparis* were discovered (8), indicating the potential capabilities of the fungus as a forest pathogen. Accordingly, the epiphytology of aerial and root infections in the native cedar region were investigated.

The host of primary concern in this study, *Chamaecyparis lawsoniana*, a timber species of exceptional quality, is known locally as Port Orford cedar or Lawson cypress. It grows naturally only along the Pacific Coast of North America—from Coos Bay, in southern Oregon, southward into Humboldt County, in northern California. The coastal shelf was formerly occupied by a mixed forest of Sitka spruce, western hemlock, Douglas fir, and Port Orford cedar. In many localities the latter was the most numerous species. This area has been logged, and Port Orford cedar, a prolific seed producer, has taken a prominent place in the regeneration. The remaining old-growth cedar is found in the more mountainous parts of the coastal range, where it occurs in stands dominated by Douglas fir.

PATHOGENICITY.—Ten morphologically indistinguishable isolates representing the geographical distribution of *Phytophthora lateralis* in Oregon were used to determine pathogenicity on cedar roots and foliage. The root dip method (7) was employed to inoculate the roots of five 3-year-old Port Orford cedar seedlings with each isolate. The plants were kept warm (18–23°C) and well watered during the entire experiment. All inoculated plants had severely infected roots when examined 6 weeks after inoculation. Most of the root systems were completely invaded and the pathogen had advanced above the soil line on the main stems. The roots of all control plants remained healthy.

The pathogenicity of the same 10 isolates was studied on Port Orford cedar foliage by placing the tips of 10 healthy, detached, foliar twigs in an unsterile, active, zoospore suspension of each isolate. After 8 hours in the zoospore suspension at 20°C the twigs were transferred to saturated humidity chambers at 20°C, where the cut stems were placed in Hoagland's mineral solution (1). After 6 days all twigs inoculated with each isolate were infected, thus confirming the extreme susceptibility of the host and the high, uniform virulence of the 10 isolates. All control twigs remained healthy.

SYMPTOMS.—Symptoms of root infection of the individual trees in a Port Orford cedar stand resembled those described for ornamentals (2, 3, 5, 6, 9). The most characteristic symptom was the uniform manner in which the entire foliar crown was affected. The earliest symptom expression of root infection was a slight wilting. Foliar color changes that followed the wilting did not appear until the base of the tree had been girdled by the pathogen. Root-infected trees showing foliar symptoms also showed symptoms of fungus invasion at the base of the tree. When the outer bark was removed the cinnamon-brown discolora-

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tion of the infected inner bark contrasted sharply with the pinkish white color of the healthy inner bark (Fig. 1). In general, the green foliar pigments were gradually destroyed and the foliage took on a bronze cast, which slowly changed to the typical light-brown color of the dead foliage. Trees killed by the root disease did not defoliate until 1–2 years after their death.

Foliar symptoms of aerial infection by *Phytophthora lateralis* were readily distinguished from foliar symptoms of the root disease (8). With a root infection, the entire foliar crown died uniformly, whereas death from aerial infections—foliar or branch—was localized and progressive. Individual aerial infections usually began on foliage hanging near the ground, and the diseased area gradually spread upward and laterally in an irregular triangle (Fig. 2).

EPIPHYTOLOGY OF AERIAL INFECTION.—*Foliar infections.*—In much of the epiphytotic region, aerial infections were associated with infested soil. Foliar infection often began in the spring where low-hanging foliage brushed against infested soil during wet, windy weather. Diseased areas were enlarged by infections from zoospores and by mycelial extension from established infections.

Aerial spread of the disease was influenced primarily by climatic conditions. The production of sporangia from newly infected cedar foliage was abundant after 36–48 hours at 10–20°C (8). Zoospores were subsequently released from these sporangia at 10–20°C, but direct germination of the sporangia by germ tubes did not occur. Since temperatures in the field at the time of foliar infections were 10–20°C, the data suggested that inoculum for aerial spread consisted mainly of zoospores.

Sporangia of *Phytophthora lateralis* usually were not air-borne. Sporangia rarely developed from infected foliage held in a saturated atmosphere with no free water present, but sporangia were abundant when infected foliage was wet. However, the mature sporangia remained attached to the sporangiophores.

Microscopic observations established that zoospores died quickly as the free water containing the zoospores evaporated from the surface of the foliage. Therefore, the minimum time required for infection by the zoospores was studied. The tips of healthy, detached, Port Orford cedar twigs were immersed in active zoospore suspensions at 20°C for various periods

**Fig. 1–2. Fig. 1.** The base of a Port Orford cedar tree (22-in. diameter) showing advanced root infection. The outer bark has been removed to show the transition between diseased (dark) and healthy (white) inner bark. The mycelium of *P. lateralis* has completely girdled the base of the tree, and the foliar crown is wilting. **Fig. 2.** Severe aerial infection of Port Orford cedar caused by *P. lateralis*. Successive zones of discoloration occur on the foliage at the periphery of such defoliated areas.

up to 12 hours. Two concentrations of zoospore suspension were used: an active dense suspension and a 1:10 dilution. After the initial exposure of zoospores, the twigs were placed in a dry atmosphere at 22°C to permit rapid evaporation from the foliar surface. The minimum time of zoospore-foliage contact required to induce foliar infections was less than 2 hours. Beyond 2 hours, infection was independent of the zoospore-foliage exposure period. The number of twigs infected increased greatly as the zoospore concentration increased. Thus, foliage was infected if viable zoospores were in contact with it for 2 hours at 20°C, and the chance of infection increased as the number of zoospores in contact with the foliage increased. As zoospores in nature are washed about in water droplets their concentrations are greatly reduced, decreasing the possibility of invasion.

Foliar infection as well as sporangial formation was influenced by temperature. Zoospores of *Phytophthora lateralis* brought about infection of detached Port Orford cedar twigs at 3–25°C. Infection at 25°C was noteworthy in that vegetative growth was very poor at that temperature, although zoospores germinated well and their germ tubes elongated rapidly (7).

The development of foliar infections from a single-tip infection was studied. Tip inoculations were made 6 feet above the soil in cedar trees having full foliar crowns extending to the ground. Inoculations were made in the spring, and development of the infections was critically observed for a year. Soon after the initial infections became established, warm, dry periods tended to restrict the development of new infections. The onset of new foliar infections was not evidenced again until December. The major development of new infections occurred in late winter and early spring. By the end of March, many new infections were apparent, and old infections were enlarged.

*Canker development.*—Established infections spread within the foliar crown also by mycelial growth back into the twigs and branches. A canker formed whenever the fungus grew from a twig into a branch. The canker soon girdled the branch, and the host tissues distal to the canker gradually declined and finally died.

Branch and stem cankers not associated with mycelial extension from foliar infections appeared to arise only when the pathogen gained entrance to the inner bark through an open wound. Attempts failed to induce canker formation by maintaining active zoospore suspensions on suberized stems.

The rate of canker development in woody stems was determined by measuring mycelial extension from a point inoculation. Stems 2 and 4 in. in diameter were inoculated by inserting pieces of mycelium into the inner bark through small wounds. The linear rate of mycelial growth up and down the stems was about 2 in. per month. Stems 2 in. in diameter were girdled in 2 months, and 4-in. stems in 5 months. This study was carried out during the cool, wet, fall and winter seasons.

After a stem had been girdled, distal growth of the mycelium was restricted, but proximal development continued, permitting the fungus to oversummer as mycelium in the inner bark of branches and stems not directly exposed to the warm sun.

*Resistant spores in host tissue.*—In the late spring, as the rainy season ended and temperatures rose, foliar infections ceased. Cankers in branches and stems continued to increase in number, but infected foliage dried rapidly. Tissue sections from infected, dried foliage revealed numerous chlamydospores and oospores of *Phytophthora lateralis* (7). These spores presumably functioned as an important oversummering stage of this pathogen. Laboratory studies have indicated that these spores germinate only after a prolonged cold period (7). Hence, resistant spores present in the infected cedar foliage during the summer months would not be expected to germinate until late fall or early winter. This was precisely the time of year when the inoculum for the aerial stage of this disease began to increase rapidly.

EPIPHYTOLOGY OF ROOT INFECTION.—The root disease of Port Orford cedar caused by *Phytophthora lateralis* occurred much more frequently and was found over a much larger region than was the aerial stage of the disease. At this phase of the epiphytotic in the *Chamaecyparis* range, economic loss from this disease was almost entirely due to root infections.

*Distribution and seasonal development of Phytophthora lateralis in the soil.*—During most of the year (May–December) the surface layers of soil in the cedar range underwent occasional and often prolonged periods of drought, and attempts to isolate the pathogen from infested soil were regularly unsuccessful. Throughout the winter and spring, however, isolations were readily made.

The distribution of the pathogen in the surface soil, between diseased and healthy trees, was studied by a selective soil isolation method, employing healthy detached cedar twigs. The twigs, about 4 in. long, were placed in and on the moist forest soil and allowed to remain undisturbed for 4 weeks. The presence of *P. lateralis* in the soil was evidenced by infection of the twigs. Uninfected twigs remained healthy and turgid. One experiment, carried out in a 50-year-old stand of cedar, involved a plot (30×30 ft.) with 3 trees diseased less than a year at one end of the plot, and 3 healthy trees at the opposite end. In November, healthy detached cedar twigs were placed in the forest soil at 6-ft. intervals throughout the plot. Periodic observations and twig replacements were made throughout the winter and early spring.

In December, active growth of the fungus in the surface soil was limited to a small area surrounding one of the diseased trees. By March the fungus was active around 2 of the diseased trees and in half the total plot area adjacent to the diseased trees. However, a few isolated points within this "infested area" did not indicate active growth of the pathogen. By April, the pathogen was actively growing around all of the diseased trees and completely covered about two thirds of the plot adjacent to the diseased trees.

In contrast to this, the pathogen was not active in the area surrounding the healthy trees.

It appeared that development of the fungus in the soil, as well as in the aerial environment, followed a seasonal cycle in which resistant spores played an important role in the oversummering stage. Inability to isolate the pathogen from the surface soil during the summer and fall, as well as the limited development of the actively growing stage of this fungus through the surface soil in the winter, indicated that the mycelium in the surface soil did not survive the dry, warm summer. Limited activity in the fall, followed by active development of the pathogen in the soil in the spring, was correlated with development of the pathogen in the aerial stage of the disease. In the winter and spring, fresh cedar litter was abundant on the forest floor, and when newly fallen foliar twigs came in contact with infested soil, they often became infected. Resistant spores (chlamydospores and oospores) developed within the infected cedar debris. The rapid build-up of soil inoculum and aerial inoculum in late winter presumably was contingent on the germination of these resistant spores. The mild temperatures and moist soil that existed in late winter and early spring were ideal for vegetative growth, enabling rapid build-up of the pathogen.

The immediate local spread of *P. lateralis* in the soil was presumably due to mycelial growth, but dissemination to new soil regions was associated with spore transport and soil movement. Soil filtrates were found to stimulate the formation of sporangia and chlamydospores in culture. The sporangia germinated indirectly to produce zoospores. Thus, the fungus, in the form of fragmented mycelium or viable spores, could be transported to new soil regions by the movement of surface water. The movement of infested soil or surface litter bearing resistant spores also facilitates dissemination of the pathogen to new areas.

*Rate of root disease development.*—The annual increase in the number of diseased trees around points of natural infection was studied in a dense stand of timber on a relatively undisturbed, uniform forest site. Port Orford cedar was the principal species in this 50-year-old stand; also included were western hemlock, Douglas fir, and Sitka spruce.

In the summer of 1954 diseased cedar trees could be seen in several localities in this stand. Six plots, each including diseased trees and surrounding healthy trees, were established to evaluate disease spread. Total area was 4 acres; included were 952 Port Orford cedar trees. The cedar trees for which records were kept were 3–30 in. in diameter, averaging 10 in. Annual increase in number of diseased trees was followed for 2½ years.

Because of the concern of the land owners over the rapid dying of cedar in this area, many diseased trees were selectively removed immediately before the plots were established. The clearing operations associated with selective cutting of dead trees disturbed the surface soil in 3 of the 6 plots, which provided a basis for comparing disease progress in disturbed areas with that in undisturbed areas.

Disease development was greater in the disturbed plots than in the undisturbed plots. The movement of soil associated with the movement of machinery and dragging of logs undoubtedly increased spread of the soil-borne pathogen over a large area. Average increase in the number of diseased trees was 7% per year in the undisturbed plots, and 13 per cent in the disturbed plots, nearly twice the natural rate.

Discussion.—The inactivity of *Phytophthora lateralis* during the warm dry summer indicates that most of the fungus mycelium exposed to the external environment is killed. However, the pathogen may live through this unfavorable period as resistant spores, as mycelium in stem and root cankers, and as mycelium in moist and deep soil.

Observations on the development of foliar infections from an initial single tip infection are noteworthy in relation to resistant spores. Foliage that becomes infected at the onset of the warm summer soon dries and the mycelium in the infected tissue produces numerous chlamydospores and oospores. The pathogen cannot be isolated from such infected dried tissue throughout the summer, but after the onset of cool moist weather in the fall the pathogen can be isolated from this tissue. In fact sporulation from the infected tissue that dried during the summer can act as the primary inoculum for foliar infection in the fall. This evidence indicates that chlamydospores and/or oospores of *P. lateralis* are resistant to high temperatures and drying conditions.

On the Oregon coast the mild rainy fall weather is similar to the spring weather, but the establishment of new aerial infections in the fall is very limited. This is due to the small production of zoospores, which are the primary inoculum for foliar infection. The pathogen continues to develop throughout the winter, but aerial infections show their greatest development during the spring. The low temperature requirements (10–20°C) of this pathogen for growth, sporulation, germination, and infection agree well with the spring nature of the disease (7).

Knowledge of the cyclic activity of this disease is of value to disease and mycological surveys. Much of the work reporting the occurrence of this and related fungi has in the past been based on sampling methods that have not given adequate consideration to the condition of the fungus in the soil at various seasons. The presence of alternating periods of activity may explain some of the inconsistencies in survey data.

In spite of the interruptions in its life cycle by the dry summers, *P. lateralis* is a fungus well adapted to survive and thrive along the coast of southwestern Oregon. On the basis of these studies the epiphytotic that now plagues native *Chamaecyparis lawsoniana* will continue to increase, seriously threatening the tree throughout its entire range.

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# MULTIPLE CLONAL TYPES OF FUSARIUM SOLANI PHASEOLI IN FIELD SOIL[1]

William C. Snyder,[2] Shirley M. Nash,[3] and Eduardo E. Trujillo[4]

SUMMARY

Field and laboratory evidence indicates that Fusarium root-rot of bean, incited by *Fusarium solani* f. *phaseoli*, is usually the result of multiple infections of the underground parts. In those naturally infested field soils studied, the fungus existed in the form of several distinctive pathogenic clones. Twenty-one such clones were recovered from one field. Thus it is apparent that Fusarium root rot of a single bean plant may result from multiple clonal infections of the fungus, in the field. That this is the case was shown by the cultural analysis of field-grown beans.

It is frequently possible to isolate as many as 6 different clones of *Fusarium solani* f. *phaseoli* (Burk.) Snyd. & Hans. during routine culturing of a single root-rot-infected bean, *Phaseolus vulgaris* L. Several questions are raised: What is the origin of such clones? Did they appear only after the plating of infected tissues on agar media? Did they originate in the tissues while the plant was still growing in the field, during the course of disease development? Or did these clones already exist in the soil, in nature, and invade portions of a given root system independently? It is known from examining diseased plants in early stages that root rot is commonly induced by multiple infections that may later blend to produce a composite disease syndrome.

Some light has recently been thrown on these questions by our studies of the population changes that take place in *F. solani* f. *phaseoli* in the field following soil treatments. Qualitative data from these studies are reported in this paper.

Methods.—The studies reported here involve a field in a coastal valley of Monterey County, California,[5] that has had a long history of bean root rot. A principal pathogen in this root rot complex is *F. solani* f. *phaseoli*. The Small White variety of dry bean is grown in rotation with other crops. The field was in spinach when the soil samples used in this study were collected.

Both composite and single samples of field soil were prepared for planting as follows: Composite samples of soil were made up from 4 collections each at 4 different locations within a field, and 5-g portions were diluted in 0.1% water agar. Aliquots of these suspensions were pipetted to agar plates, and the suspension was spread over the entire surface of the agar by rotating and tilting the plates. The moisture from the suspension was absorbed by the agar in a few hours, leaving the soil distributed in an even film over its surface. The plates were incubated in diurnal light. Single soil samples were made up from each location. A 50-mg portion of the soil was diluted in 10 ml of the 0.1% water agar and spread over 10 agar plates.

The medium used was a modification[6] of Martin's agar. Plates were poured and allowed to stand 2–3 days before use. Colonies of *F. solani* f. *phaseoli* that appeared on the dilution plates were sporulating after 5–7 days and recognizable as to species by microscopic examination. A plate showing such colonies is illustrated in Figure 1.

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[5] The writers acknowledge the valuable assistance of Roy Bardin, Plant Pathologist in the Office of the Agricultural Commissioner, Monterey County, in carrying out the field program.

[6] Modification of Martin's agar medium: 15 g peptone, 0.5 g $MgSO_4 \cdot 7H_2O$, 1 g $KHPO_4$, 25 g agar, 1000 ml water, and 1/30,000 parts rose bengal. After making up the medium it was placed in bottles in 100-ml lots and autoclaved. At the time of pouring plates, 10 drops of 10% sodium taurocholate and enough Streptomycin to give 300 ppm were added to each bottle.

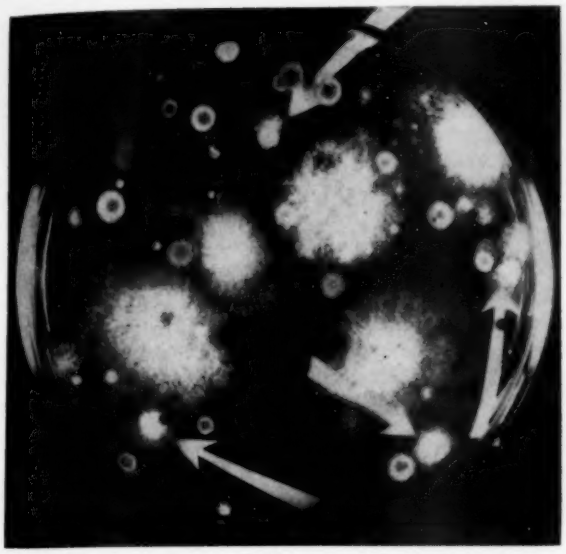

FIG. 1. A dilution of field soil cultured on a plate of modified Martin's medium. Arrows indicate colonies of *Fusarium solani* f. *phaseoli*.

RESULTS.—From 36 dilution plates made from soil suspension of 4 composite samples, 178 colonies were identified as *F. solani*, and of these 120 were tentatively identified as *F. solani* f. *phaseoli*. The total amount of field soil plated in these dilutions was 133 mg, on a dry-weight basis, and is representative of the 60 g of field soil originally composited and from which the dilutions were ultimately prepared.

Tentative diagnosis of the pathogen as *F. solani* f. *phaseoli* was confirmed as follows: Five single-spore transfers were made directly to potato-dextrose slants from each of the 120 colonies detected as being the pathogen on the soil dilution plates. A comparative cultural examination of these transfers showed that 20 distinct clones were present, representing the 120 original colonies. The 20 clones were then individually tested for pathogenicity on healthy bean plants grown in the greenhouse. Each clone was tested in triplicate, three pots, 4 plants to a pot. Of the 20, only 1 was non-pathogenic; all the others were pathogenic but differed in virulence. A comparison of clones isolated from soil with clones isolated from infected bean plants from the field showed that the commonly isolated clones from the two sources were the same.

Twenty plates were made of the two 50-mg field soil samples, and the 56 colonies obtained were tentatively identified as *F. solani* f. *phaseoli*. Of these, 29 were single-spored and tested in the same way as the isolates from the composite soil samples. From these, 15 distinct clones were identified, and, in pathogenicity tests, all proved to be *F. solani* f. *phaseoli*. Two of the clones were quite different from the 19 identified in the preceding tests. Eight are shown in Fig. 2. The number of clones present among the 27 colonies of *F. solani* f. *phaseoli* isolated from the other 50-mg soil sample was not determined.

An average of 895 units of *F. solani* f. *phaseoli* were recovered per gram of the composite samples of field soil, and an average of 690 colonies per gram of the single soil samples collected from the same field one month later. There appeared to be little difference whether the soil samples were collected at the ground surface or at a depth of 6 in., possibly because the field was kept moist by winter rains. By actual single spore, comparative examinations and pathogenicity tests, a total of 175 mg of field soil (on a dry weight basis) yielded 21 distinct clones (19 from the first

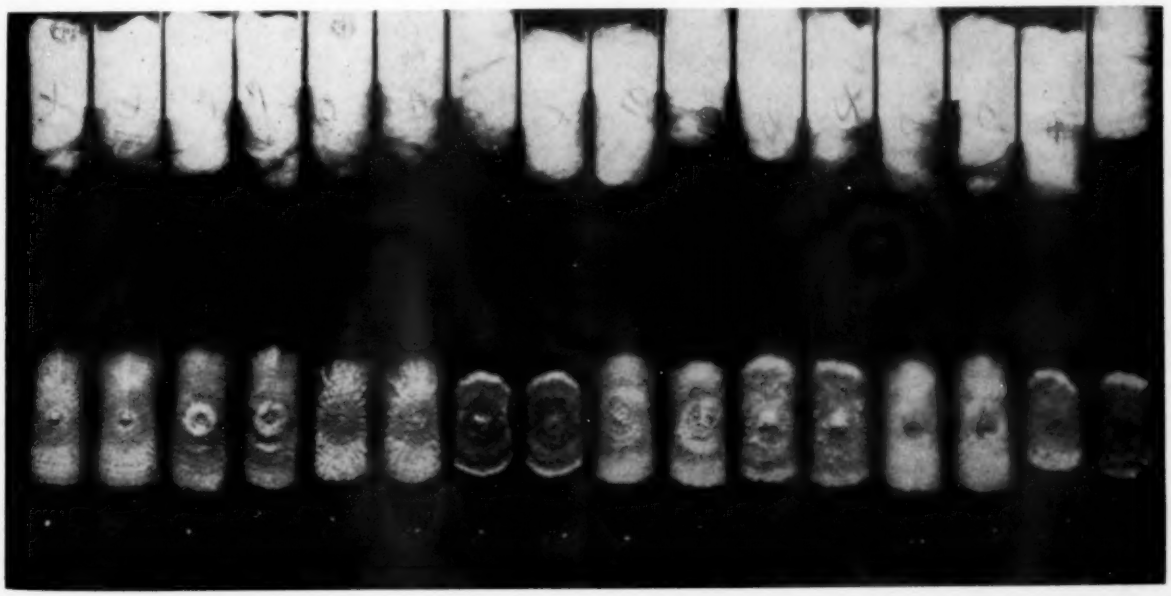

FIG. 2. Representatives of 8 of 15 different clones, photographed in pairs 2 weeks after they were single-spored, found among 29 colonies of *F. solani* f. *phaseoli* obtained from a culture of 1 50-mg sample of field soil on dilution plates.

tests, 2 additional ones in the second test) of *F. solani* f. *phaseoli*. Subsequent collections and platings from other root-rot-infested fields in the area gave similar results.

The composite samples reflect the quality of the clonal types present in the field as a whole, while the single samples indicate that diverse clonal types may occur in any one location in the field.

Discussion.—The results show rather conclusively that the soil population of *F. solani* f. *phaseoli* in the particular field in question is composed of a number of distinct clones as measured by differences in growth, appearance in pure culture, and virulence. This is similar to the range of clonal types of the mimosa wilt Fusarium reported to occur in nature.[7] It seems beyond question, therefore, that multi-clonal infections of individual bean plants originate directly from the exposure of underground parts of the bean to clones of the root rot Fusarium already in existence in natural soil. This conclusion is based upon a) recovery of several clones of the fungus from diseased individual plants taken from the field, and b) direct isolation from soil of these same and additional distinct clones verified by cultural and pathogenicity studies. Soil population studies revealed the presence of at least 21 different clones per composite gram of field soil. The data seem even more striking when it is realized that the soil samples were taken during the winter growing season, when the field was in spinach, some 4 and 5 months after the preceding bean crop had been harvested.

Root systems of spinach plants growing in the field were examined visually and by culture for any possible relationship to the Fusarium. The fungus was not detected in or on the roots.[8] Furthermore, the dilution plate medium was tested for its mutagenic properties toward the Fusarium by placing several homocaryotic clones on the medium and then single-sporing to potato-dextrose agar. No evidence was obtained that the isolation medium influenced the mutation rate of the Fusarium within the periods involved in the soil culture experiments. Moreover, the differences between clones of the fungus are detectable in the colonies on the dilution plates as they develop.

It is widely recognized that a fungus such as *F. solani* f. *phaseoli* may show a broad range of diversity in culture in the laboratory. It is perhaps not so widely recognized that such a fungus may occur in naturally infested field soil in a highly diverse condition. It is now apparent that the recovery of different clones from a single root-rot-infected bean plant may reflect the pathogenic activities of multiple-clonal wild types of the Fusarium in the soil.

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[8] A cultural examination of the spinach roots was made by Floyd F. Hendrix.

# THE EFFECT OF AMINO ACIDS ON SUSCEPTIBILITY OF APPLE VARIETIES TO SCAB[1, 2]

J. Kuc, E. Barnes, A. Daftsios, and E. B. Williams[3]

SUMMARY

The D and DL isomers of phenylalanine markedly increased the resistance of 7 apple varieties to attack by the apple scab incitant *Venturia inaequalis*. A solution of the amino acid was infused into growing shoots through leaf midribs for a 24-hr period, and leaves above points of infusion were then inoculated with a conidial suspension of *V. inaequalis*. Leaves were examined for the appearance of symptoms 10–21 days after inoculation. The number and size of sporulating lesions were markedly reduced on 5 commercial varieties, Golden Delicious, McIntosh, Jonared, Yellow Transparent, and Gallia Beauty infused with D- or DL-phenylalanine. The commercial varieties are susceptible to races 1, 2, and 3 of *V. inaequalis*, and infusion did not result in a resistant reaction but merely a reduction in the degree of susceptibility. After infusion with D- or DL-phenylalanine, selections of the Russian variety R12740-7A susceptible to race 2 and resistant to races 1 and 3 of *V. inaequalis*, and selections of the variety Geneva susceptible to race 3 and resistant to races 1 and 2 were found resistant to all three races. Small pin-point pits or non-sporulating necrotic or chlorotic areas, typical of a resistant reaction, were evident on these varieties instead of the susceptible reaction. The L, or naturally occurring, isomer of phenylalanine did not alter the disease reaction of the 7 varieties tested. Solutions of D-alanine, DL-α-aminobutyric acid, and, to a lesser degree, D- and DL-leucine, infused only into the Russian variety, had an effect similar to that of D- or DL-phenylalanine; whereas DL- or L-alanine, L-leucine, DL-, D-, or L-tyrosine, DL-, D-, or L-dihydroxyphenylalanine, and DL- or L-cysteine hydrochloride had no effect. The DL, D, and L isomers of dihydroxyphenylalanine appeared inhibitory *in vitro* to several isolates of race 2 of *V. inaequalis*; however, the other amino acids were not appreciably inhibitory *in vitro* at concentrations equal to those used for infusion.

INTRODUCTION.—Apple scab is incited by the ascomycetous fungus *Venturia inaequalis* (Cke.) Wint. The organism attacks the leaves, flowers, and fruit of apple. Kirkham (2) found that water-soluble plant phenols may be strongly inhibitory to the growth and sporulation of *V. inaequalis*, depending on the amount of nitrogen and its source. Infusing apple leaves with the phenolic fraction extracted from Cox's Orange Pippin reduced infection, whereas urea infused with the phenolic fraction negated this effect, and urea alone stimulated infection. Infusions produced their maximum effect on leaves approaching the mature resistant stage. It is thought that the aromatic nitrogen ratio at this stage of leaf development can be most effectively altered by infusion. Induced biochemical mutants have recently been used by Boone, Kline, and Keitt (1) and Kline, Boone, and Keitt (3) as a tool in a study of the pathogenicity of *V. inaequalis*. Mutants requiring, respectively, choline, riboflavin, purines, pyrimidines, arginine, histidine, methionine, or proline were found to be nonpathogenic. Pathogenicity was restored to mutants requiring choline, riboflavin, pyrimidine, arginine, histidine, and methionine by daily application of the required substance to inoculated leaves. Pathogenicity could not be restored to 2 mutants, one requiring either adenine or hypoxanthine, and one requiring either guanine or xanthine.

MATERIALS AND METHODS.—*Infusion and inoculation of trees.*—Apple trees 1–2½ feet tall of the varieties Russian (*Malus pumila* R12740-7A), Geneva (*M. pumila* var. *niedzwetzkyana* o.p.), Golden Delicious, McIntosh, Yellow Transparent, Jonared, and Gallia Beauty (*M. pumila*) were used in tests. Trees were grown under greenhouse conditions in 6-in. pots containing soil at 60–75°F. The first fully opened leaf was designated as leaf 1, and liquids were infused into lower leaves 2, 3, 4. Leaves were prepared for infusion by cutting off most of the blade, leaving the midrib surrounded by about 1–2 mm of blade tissue. The tip of the midrib was then cut, and the midrib and part of the petiole were placed immediately in a 1½-dram shell vial containing the amino acid. Vials were refilled after about 6 hours; total uptake of solution was 4–8 ml in 24 hours. The vials were then removed, and the leaves above the point of infusion were inoculated with a suspension of conidia of *V. inaequalis* containing about 100 conidia per low-power (100×) field of a microscope. Plants were kept in a moist chamber for 48 hours after inoculation. Races of *V. inaequalis*, as reported by Shay and Williams (4), were used for inoculation. On the Russian variety races 1 and 3 produce typical resistant reactions, irregular or regular necrotic or chlorotic lesions, and no sporulation, and race 2 produces a typical susceptible reaction, extensive lesions

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[2] Research supported in part by grant No. G1811 of the National Science Foundation.

[3] All of Purdue University, Lafayette, Indiana. Respectively, Assistant Professor, Department of Biochemistry and Department of Botany and Plant Pathology; Graduate Fellow, Department of Botany and Plant Pathology; Graduate Assistant, Department of Biochemistry; and Associate Professor, Department of Botany and Plant Pathology.

TABLE 1.—*Susceptibility to Venturia inaequalis of Russian and Geneva apple trees infused with various amino acids*

| Variety and materials injected | Concentration | Reaction after 10–21 days[a] Race 1 | Race 2 | Race 3 |
|---|---|---|---|---|
| Russian[b] | | | | |
| DL-phenylalanine | $3\times10^{-2}M$ | 1&2 | 2 | 1&2 |
| D-phenylalanine | $3\times10^{-2}M$ | 1 | 1 | 1 |
| L-phenylalanine | $3\times10^{-2}M$ | 2 | 4 | 2 |
| DL-,L-alanine | $3\times10^{-2}M$ | 2 | 4 | 2 |
| D-alanine | $3\times10^{-2}M$ | 1 | 2 | 1 |
| DL-,D-leucine | $3\times10^{-2}M$ | 2 | 2&3 | 2 |
| L-leucine | $3\times10^{-2}M$ | 2 | 4 | 2 |
| DL-,D-,L-tyrosine | $2\times10^{-3}M$[c] | 2 | 4 | 2 |
| DL-,D-,L-dihydroxyphenylalanine | $1\times10^{-2}M$[d] | 2 | 4 | 2 |
| DL-,L-cysteine hydrochloride | $2.5\times10^{-2}M$[d] | 2 | 4 | 2 |
| DL-α aminobutyric acid | $3\times10^{-2}M$[e] | 0 | 1 | 0 |
| water | | 2 | 4 | 2 |
| Geneva[b] | | | | |
| DL-phenylalanine | $3\times10^{-2}M$ | 1&2 | 1&2 | 2 |
| D-phenylalanine | $3\times10^{-2}M$ | 1&2 | 1&2 | 1&2 |
| L-phenylalanine | $3\times10^{-2}M$ | 2 | 2 | 4 |
| water | | 2 | 2 | 4 |

[a] Resistant reactions: 0 = no macroscopic evidence of infection; 1 = minute pits, no sporulation; 2 = irregular or regular necrotic or chlorotic lesions, no sporulation. Susceptible reactions: 3 = restricted necrotic lesions with sporulation, no defoliation; 4 = extensive lesions with abundant sporulation, infected leaves may abscise.

[b] The Russian variety is resistant to races 1 and 3 and susceptible to race 2 of *V. inaequalis;* the Geneva variety is resistant to races 1 and 2 and susceptible to race 3.

[c] Concentration limited by the low solubility of tyrosine in water.

[d] Higher concentrations produce severe phytotoxicity.

[e] Some evidence of abnormal growth—twisting of leaves and shoots.

with abundant sporulation, and occasional abscission of infected leaves. On Geneva, races 1 and 2 produce resistant reactions and race 3 a susceptible reaction. The five commercial varieties are susceptible to all isolates of races 1, 2, and 3 used in this study.

*In-vitro studies.*—The following amino acids were tested: DL, D, and L isomers of phenylalanine, alanine, leucine, tyrosine and dihydroxyphenylalanine, DL- and L-cysteine hydrochloride, and DL-α-aminobutyric acid. Solutions of the amino acids were prepared at twice the desired concentration and passed through sterile glass bacterial filters. The solutions were then each mixed with an equal volume of sterile warm PDA[4] and adjusted to pH 5.5. About 16 ml of the resultant medium was poured per Petri plate, and plates were seeded by pipetting 0.5 ml of a heavy suspension of conidia of *V. inaequalis* over the agar surface. Plates were held at 70°F, and growth observed after 14 days.

[4] Prepared by dissolving twice the required concentration of dehydrated PDA in water and autoclaving 15 minutes at 15 lb pressure. The concentration of PDA after addition of the amino acid solution was 4 g per 100 ml of medium.

RESULTS.—*Effect of amino acids on reaction of host.*—The respective disease reactions of Russian to race 2 and Geneva apple trees to race 3 of *V. inaequalis* were altered from susceptibility to resistance after infusion with D- or DL-phenylalanine (Table 1). Pits and necrotic or chlorotic lesions were evident on the leaves instead of the normal susceptible reaction. The resistance of Russian trees to races 1 and 3, and of Geneva to races 1 and 2, changed after infusion with D- or DL-phenylalanine, from necrotic or chlorotic lesions to pits. In contrast to the effect on Russian and Geneva varieties, D- and DL-phenylalanine infused into the commercial varieties reduced the number and size of sporulating lesions but did not alter the type of reaction (Table 2). Infusion of trees with L-phenylalanine, the naturally occurring isomer, or water did not influence the reaction of any of the varieties tested to the three races of the fungus. Infusion into Russian trees of D-alanine, DL-α-aminobutyric acid, and, to a lesser degree, D- or DL-leucine had the same effect as D- or DL-phenylalanine, whereas DL- or L-alanine, L-leucine, D-, DL-, or L-tyrosine, D-, DL-, or L-dihydroxyphenylalanine, and DL- or L-cysteine hydrochloride had no effect on symptom expression (Table 1). Alanine, α-aminobutyric acid, cysteine hydrochloride, dihydroxyphenylalanine, leucine, and tyrosine were infused only into the Russian variety.

*Growth of the fungus on media containing amino acids.*—*V. inaequalis* grew well on the PDA medium and on the media containing the amino acids, except DL-, D-, or L-dihydroxyphenylalanine, at concentrations equal to those used for infusion. Dihydroxyphenylalanine markedly inhibited the growth of several isolates of race 2, but had no effect on the isolates of races 1 and 3.

DISCUSSION.—The differences in response of the apple varieties to infusion with D- and DL-phenylalanine further suggest the presence of a mechanism for resistance to *V. inaequalis* in Russian and Geneva, and its absence or low activity in the commercial

TABLE 2.—*Susceptibility to Venturia inaequalis of commercial apple varieties[a] infused with phenylalanine ($3\times10^{-2}M$)*

| Injectant | Degree of susceptibility[b] Mc-Intosh | Jonared | Golden Delicious | Yellow Transparent | Gallia Beauty |
|---|---|---|---|---|---|
| DL-phenylalanine | + | + | ++ | + | ++ |
| D-phenylalanine[c] | + | 0 | + | 0 | + |
| L-phenylalanine | +++ | +++ | +++ | ++ | ++ |
| water | +++ | +++ | +++ | ++ | +++ |

[a] Varieties listed are susceptible to the three races of *V. inaequalis.*

[b] 0 = no macroscopic evidence of infection; + = scattered and restricted sporulating lesions; ++ = sporulating lesions covering less than 50% of leaf area; +++ = heavily sporulating lesions covering more than 50% of leaf area. Averages for 10–18 trees per injectant per variety. Trees showing severe injury were not included in the averages.

[c] Phytotoxicity evident on some trees, especially on Jonared and Yellow Transparent.

varieties. It appears that a mechanism for resistance of the Russian and Geneva varieties to races 1, 2, and 3 can be activated by D- and DL-phenylalanine, regardless of the race of the fungus used for inoculation. This mechanism appears lacking or decreased in effectiveness in varieties susceptible to the three races of the fungus (commercial varieties), and infusion of these varieties with D- or DL-phenylalanine does not result in a resistant reaction. The decrease in susceptibility in the commercial varieties suggests a mechanism capable of modifying only the degree of susceptibility. This mechanism is perhaps operative in nature, and would be active in determining the differences in degree of scab-susceptibility exhibited by different commercial varieties. The ultimate reaction of host to fungus is highly specific. The activation of metabolic pathways responsible for susceptibility, degree of susceptibility, or resistance must depend on the pathways available in the host, and on how they are affected by the different races of the fungus.

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# REPORT AND ABSTRACTS OF THE 1959 ANNUAL MEETING OF THE SOUTHERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, FEB. 2-4, 1959

The Southern Division of the American Phytopathological Society held its annual meeting in conjunction with the 56th annual convention of the Association of Southern Agricultural Workers, at Memphis, Tennessee, February 2-4, 1959. About 130 plant pathologists attended, 34 papers were presented. At the business meeting on February 2 it was announced that the following officers had been elected by mail ballot: President, E. M. Cralley; Vice-President, W. J. Martin; Councilor, W. W. Hare. Appointed as secretary-treasurer for a term of 3 years was J. P. Fulton.

W. J. MARTIN
*Secretary-Treasurer*

*Studies on the biology of the stem nematode.* BARKER, KENNETH R. Thirty-six plant species and vars. were inoculated with stem nematode (*Ditylenchus dipsaci*) larvae from alfalfa from Wake and Haywood Counties, North Carolina. Both populations reproduced readily in alfalfa (vars. Atlantic, Buffalo, and Du Puits), soybean (var. Lee), bean (var. Wade), and reproduced slightly in resistant alfalfa (vars. Lahontan and Talent), red clover (var. Kenland), Austrian winter pea, burley tobacco (Ky. 16), tomato (var. Marglobe), and rye. Slight infection occurred in 11 other species and vars., and 12 species were not infected. On Wando garden pea, the Wake Co. population caused swelling and multiplied extensively, but the Haywood Co. population induced necrosis of the apical bud and did not reproduce. The N.C. populations, 1 from alfalfa from Nevada, and 1 from fullers teasel from Washington, were compared on 9 plant species (Seinhorst 1957). The teasel strain did not infect alfalfa, and alfalfa strains failed to reproduce on teasel. No morphological differences were found in the alfalfa strains, but the teasel strain was generally larger. Necrosis appeared in a high percentage of the resistant plants, suggesting a hypersensitive reaction. Invaded areas stained deeply with safranin and contained few nematodes. Both N.C. populations reproduced to a greater extent at 65°F than at 55° or 75°. High moisture increased infection and reproduction at all 3 temperatures.

*Loss measurements caused by the bacterial blight disease of cotton.* BIRD, L. S. The split-plot experimental design was used to compare resistant and susceptible cotton strains under controlled infection caused by *Xanthomonas malvacearum*. Artificial inoculation was used to establish and maintain the disease in the blight-infected plots. Experiments were conducted at 2 locations in 1952 in the presence of races 1 and 2 of the causal organism. No boll infection resulted; therefore, the loss obtained was due to leaf infection only. The yield-loss range was 14–32% in one test and 9–34% in another test. The degree of loss was associated with the level of tolerance in the cotton strains. A significant reduction in lint per cent from the disease was measured in one test. A single 1958 test was conducted in the presence of race 1 of the causal organism. The yield was significantly reduced 6.4% by leaf infection. A significant reduction in weight per 100 seed from the disease was also measured. By observation, the per cent reduction in yield due to leaf infection is about ½ the per cent of leaf drop caused by the disease.

*Nematospora boll rot of cotton found in Texas.* BLACKMON, CYRIL W., AND L. S. BIRD. A yeast was isolated from bolls with a certain distinctive type of boll rot collected in the Waco area in 1957. This type of rot was first noticed in 1952 on the Lubbock station. A survey made in 1958 indicated that the yeast boll rot was distributed throughout the state. Careful comparison of isolates from the rotted bolls with *Nematospora coryli* (ATCC 10647) and Ashbey and Nowell's published description of *N. gossypii* definitely established that the isolates were *N. gossypii*. A check of the literature revealed no instance of *N. gossypii* having been reported on cotton in the U.S. *N. coryli* has been reported on cotton in California. Inoculations made by puncturing the boll wall with toothpicks were about 10% effective. The organism causes a brown fiber stain. In severe instances it may completely digest the fiber, leaving naked seed in the bolls.

*The effect of certain treatments on the germination of sclerotia of Sclerotium rolfsii.* Boswell, T. E. Freshly matured sclerotia from pure cultures of *S. rolfsii* were subjected to various treatments to determine their effect on germination. Highly significant increases in percentage germination were obtained by removing more than 75% of the moisture from sclerotia with a Brabender Moisture Tester operating at 98°F. Results were similar when the sclerotia were dried at 120°F; however, exposures of sclerotia to this temperature for 2.5 hours or more was injurious to germination. Scarifying sclerotia of *S. rolfsii* with 220-mesh carborundum for 30 minutes or with 400-mesh carborundum for 60 minutes resulted in 100% germination. Soaking sclerotia 3 minutes in a solution of either 0.5% sodium hypochlorite or 3% hydrogen peroxide produced significant increase in germination over that obtained from untreated sclerotia. Leaching the sclerotia with distilled water for 48 hours improved germination of older sclerotia, but was not effective with freshly matured sclerotia. Soaking in distilled water for 48 hours gave a highly significant increase in germination of both freshly matured and older sclerotia. These studies indicate that there is a type of dormancy in sclerotia of *S. rolfsii* that apparently is inherent in the sclerotial coating.

*The effect of certain pre-storage and storage treatments at various relative humidity levels upon germination of sclerotia of Sclerotium rolfsii.* Boswell, T. E. Freshly matured sclerotia from pure cultures of *Sclerotium rolfsii* were stored for 7–63 days in jars in which RH (relative humidity) was controlled at various levels of 0–100%. The highest germination (75–90%) and most vigorous growth occurred after storage for 7–16 days at RH levels of 25–35%. Notably lower germination (11.7–58.3%) and less vigorous growth occurred where sclerotia were stored at RH levels of 50–95% for the same period of storage. However, the best germination of sclerotia occurred after 23–61 days' storage at the higher RH levels. The reverse occurred when the sclerotia were dried at 98°F for 10.5 hours before being placed in the humidity chambers. Sclerotia that were surface-sterilized in a 10% Clorox solution for 3 minutes before storing at RH levels of 15–95% gave an average germination of 86.4–99.7% for all 6 germination tests. Germination of sclerotia stored at the 0.0% RH level averaged only 3.6%. The 100% RH level was not satisfactory for storing the NaOCl-treated sclerotia, because most of the sclerotia germinated in the humidity chamber within 12–14 days.

*Sting nematode control on four crops with pre- and post-plant applications of Nemagon.* Cooper, W. E., J. C. Wells, and J. N. Sasser. The effectiveness of Nemagon (1,2-dibromo-3-chloropropane), 17.3% granular, as a control of the sting nematode, *Belonolaimus gracilis*, was evaluated at 4 rates in pre- and post-plant applications on peanuts, corn, soybeans, and cotton. Pre-plant applications were applied in the row 2 weeks before planting, and post-plant side dress applications 1 and 2 months after planting. Rates tested were 0.0, 0.5, 1.0 and 1.5 gal./A. of technical fumigant replicated 4 times in 25-ft. single-row plots. Pre-plant applications gave increased growth and yield for all crops and were superior to post-plant treatments. Greatest yields from the pre-plant treatment of cotton were obtained at 1 and 1.5 gal./A. Sting nematodes had killed most of the cotton seedlings by the time of the first post-plant treatment. Corn yields were greatest with the 1 gal./A. pre-plant treatment, which increased yield 93% over the 58 bu./A. yield of untreated plots. The post-plant treatments of corn gave no increase in yield. All pre-plant treatments increased the yield of peanuts and soybeans over untreated controls. Post-plant treatments of these crops also gave increased yields over untreated plots. This was more pronounced in the first post-plant treatments. In general, nematode control was correlated with dosage and crop performance.

*A method for quantitative mold determination in peanuts.* Diener, Urban L. A 100-gram sample of unshelled peanuts was selected from a large lot by means of a riffle. After hand-shelling, the sample consisted of 50 g, or about 100 kernels. It was pulverized in a Universal "71" food chopper or a No. 30 Chop-O-Matic, depending on seed moisture content. Ten g of ground peanuts were placed in a 400-ml beaker with 200 ml of water and homogenized 6 minutes in a Bodine Centrifugal Wet Mill. This sample was diluted from 1:20 to 1:200, 1:2000, and 1:20,000, using 10-ml aliquots and 90 ml of water. One-ml aliquots of each dilution were pipetted into each of 3 Petri dishes, and 10–12 ml of malt-salt agar (50–52°C.) were then added. Cultures were incubated at 25°C. after cooling. Mold counts were made in 4–5 days, and again at 8–10 days with a stereoscopic microscope (10×). Analysis of the data of several experiments revealed that the number of independent samples from a lot required to define a stated difference in mold count for 40% of the mean was 2 or 3. The necessary sample size was determined to be 20-50 g of shelled peanuts.

*Mold determination in stored farmer-stock peanuts.* Diener, Urban L. Peanuts stored in farm-size bins of the Commodity Credit Corporation at the Wiregrass Substation, Headland, Alabama, were sampled randomly on June 5, 1957. Two independent 10-lb. samples were taken from the surface and from the middle of the bin with a probe. Quantitative mold counts were made on 100 g of unshelled peanuts riffled from these samples by a method previously reported. The predominant fungi were 5 species of the *Aspergillus glaucus* group (*A. ruber, A. repens, A. chevalieri, A. restrictus, A. amstelodami*), *A. tamarii*, and *Penicillium citrinum. P. funiculosum, P. janthinellum, Cladosporium herbarum*, and *Torula sacchari* occurred frequently. Thirteen other fungus species, 6 of them *Aspergilli*, occurred less commonly. The mold count of samples ranged from 7 to 1,350,000 colonies per g of peanuts. High mycofloral counts were associated with high initial moisture. There was no relation between high mold count and high initial damage, storage-type of bin, or years in storage, that was not associated with high initial moisture. In some instances, high mold counts were associated with an increase in damage during storage.

*Is the quality of small grain seed improved by cleaning and treating?* Earhart, R. W. It is accepted by the seed trade and most growers that cleaning and treating will improve the quality of small grain seeds. Data developed in the pathological analysis of small grain seeds collected in a state-wide survey in South Carolina indicate that, though processing does improve seed quality, there are many exceptions. Pathological analysis of the cleaned and treated seed from 15 counties indicates an average disease index value of 6.3 (disease indices based on a 0–100 scale, where 0 equals no visible symptoms), whereas the field run seed from these same counties averaged 21.1. However, among these 274 cleaned and treated seed lots there was one with a disease index of 65.8, and 54 (19.7%) with disease indices so high that they appeared untreated. Our results show that seed processors are doing an effective job much of the time, but that most of them had coming through their processing plants occasional seed lots that were not effectively processed. This indicates that seed processors need additional or improved equipment, or need closer supervision of processing operations.

*Response of root-lesion nematodes, Pratylenchus brachyurus and P. zeae, to various host plants and soil types.* Endo, Burton Y. Thirty-three plant species and varieties were tested as to relative suitability for infection and reproduction of *Pratylenchus zeae*. Of these, 4 were classified as very favorable, 6 as favorable, and 23 as not favorable. Of 30 plant species and varieties tested with *P. brachyurus*, none were classified as very favorable, 9 as favorable, and 21 as not favorable. Reproduction of *P. brachyurus* on

host plants over 5 months indicates that large populations can be obtained on corn and peanut plants, whereas very small populations were associated with oats and crotalaria. Norfolk sandy loam was found to be the most favorable soil type for the infection and reproduction of these lesion nematodes on suitable host plants; Cecil clay loam was the least suitable, and Portsmouth loam was intermediate. Migration of root-lesion nematodes was influenced by soil type and the presence of a suitable host plant. Maximum migration of 5 in. in 4 months occurred in Norfolk sandy loam planted to corn. Shortest migration was recorded for Cecil clay loam, whereas intermediate distance was noted for Portsmouth loam. In the absence of host plants, little or no migration occurred in any of the soil types.

*Control of peanut stem rot in Virginia by cultural practices.* GARREN, K. H. A 4-year intensive study of stem rot of Virginia Bunch peanuts caused by *Sclerotium rolfsii* has been completed in Virginia. The effects on the disease of controlling weeds with dinoseb (DNBP herbicide) + cultivation that threw no soil onto the plants was compared with controlling weeds with cultivation that threw soil onto the plants. Both practices were superimposed on land preparation resulting in deep and shallow covering of surface litter. A high degree of disease control was achieved each year. Infection was consistently less when no soil was thrown onto plants than when it was. In 2 years when infection was heavy, deep covering of surface litter gave better results than shallow covering. In a comparison test when infection level was high the independent beneficial effects of not throwing soil onto the plants were much greater than those of the dinoseb. PCNB (pentachloronitrobenzene) applied 2 ways also had beneficial effects, but these never equaled the independent beneficial effects of not throwing soil onto the plants.

*Evaluation of methods for applying 1,2-dibromo-3-chloropropane for controlling root-lesion nematodes on spanish peanuts.* GOOD, J. M., and A. E. STEELE. Root-lesion nematodes, *Pratylenchus brachyurus*, have been associated with peg and pod rot of peanuts, but the economic importance of this pest on peanuts has not been clearly demonstrated. To obtain additional information of the damage caused by *P. brachyurus* to peanuts, DBCP (1, 2-dibromo-3-chloropropane) was applied as preplanting row and broadcast applications and as a post-planting sidedressing when the first blooms appeared. A liquid formulation of DBCP was injected with tractor-drawn equipment at ½ gal. per acre (for 38-in. row spacing) in the row for the preplanting row treatment, and on both sides of the row for the post-planting sidedressing treatment. The broadcast treatment was a surface application of 10% granular DBCP, cut-in with a disk-harrow at a rate equivalent to 2 gal./A. of technical DBCP. As compared with no fumigation, the 3 methods of applying DBCP gave significant and satisfactory control of *P. brachyurus*, greatly reduced incidence of peg and pod rot, an average yield increase of 20.4%, an average of 6.2% fewer pods left in the ground at harvest, and a higher market grading price for pod samples. The preplanting row application of DBCP was superior to the broadcast and sidedressing applications.

*Contact fungicides as late dormant sprays for pecan scab control.* GRAVES, CLINTON H., JR. Field tests were carried out for 2 consecutive years in nursery plots and permanent orchards of Stuart and Mahan pecans for the purpose of eliminating the main source of overwintering scab inoculum —the holdover stromata on infected twigs. Only materials that appeared promising in laboratory and prior tests were considered: Santobrite (sodium pentachlorophenate), Corona CM-220 (monocalcium meta-arsenite), and two quaternary nitrogen compounds—Puratized Agricultural Spray (phenylmercuritriethanol ammonium lactate) and Puratized Apple Spray (phenylmercurimonoethanol ammonium acetate). Of these, the quaternary nitrogen compounds (5 pints per 100 gal.) consistently gave the best results, followed by Santobrite (4 lbs. per 100 gal.). The dormant application of the quaternary nitrogen compounds gave a highly significant disease decrease at midseason, but only when followed by proper application of regular early-season sprays. Santobrite also gave a significant decrease. Where the tests were not followed by the regular season sprays, only the quaternary nitrogen compounds showed a slight, though significant, decrease by mid-season. Nursery plots were more responsive than permanent orchards.

*Breeding tobacco-etch-virus-resistant Tabasco-type peppers.* GREENLEAF, W. H. A program for breeding etch-virus-resistant Tabasco-type peppers has been conducted since 1953, using the resistance discovered in P.I. 152225 by S. G. Younkin in 1949. Breeding has reached the state: ($F_3$ (Tabasco $\times$ $F_2$ (P.I. $\times$ Tabasco)) $\times$ Tabasco. Prospects appear good for the development of a commercially desirable etch-resistant Tabasco type that will be superior to this variety in certain other characteristics. This development is timely in view of a serious wilt disease of Tabasco caused by the etch virus that is threatening the industry built on this variety in South Louisiana.

*Factors influencing the emergence of larvae from cysts of Heterodera glycines Ichinohe. Cyst development, condition, and variability.* HAMBLEN, M. L., and D. A. SLACK. In greenhouse and laboratory studies, development of cysts and emergence of larvae from cysts were influenced by the moisture condition of soil in which soybean plants were growing prior to collection of cysts. A higher percentage of brown cysts and significantly greater emergence of larvae from brown cysts was correlated with moisture stress on soybean plants during a 2-week period preceding the recovery of cysts from soil. Adequate or excessive soil moisture for growth of soybean plants resulted in a high percentage of so-called white or yellow cysts as compared to brown cysts, and less emergence of larvae from brown cysts recovered from the soil. The degree of cyst maturity and the condition of brown cysts influenced the number of larvae that emerged. In decreasing order of the number of larvae that emerged, cyst designations were: 1) white cysts, 2) brown floaters that would not sink in water, 3) yellow cysts, and 4) brown sinkers. Although the total number of larvae that emerged from a collection of brown cysts was frequently high, considerable variability occurred in larval emergence from single selected cysts. In several tests, about 90% of the total emerged larvae were attributable to 10% of the cysts. Rupture of the brown cysts following the tests revealed numerous eggs with developed larvae, and some hatched larvae in all cysts.

*Fungi isolated from white clover and their associative effects upon Sclerotium rolfsii.* HANSEN, J. D., and E. A. CURL. During 1957 and 1958 the effects of some fungi associated with *Sclerotium rolfsii* were studied. *S.-rolfsii*–infected stolons and healthy stolons of *Trifolium repens* were collected from 4 experimental fields. Each diseased stolon was arbitrarily divided into 3 zones designated as dead tissue, discolored but living tissue, and green tissue. All stolons were washed thoroughly, and fungi were isolated from the 3 zones of diseased stolons and from cuttings of apparently healthy stolons. Isolates of the organisms were tested for antagonistic or stimulatory effects upon *S. rolfsii* and one another. The predominant fungi isolated from each of the 3 zones of diseased stolons were *Fusarium* spp. and *S. rolfsii*. Other fungi frequently isolated were species of *Mucor*, *Trichoderma*, *Colletotrichum*, *Geotrichum*, and an unidentified fungus. *Fusarium* ssp., *Colletotrichum* ssp., and the unidentified isolate were more prevalent in the green zones of the infected stolons and in apparently healthy stolons than in other zones. The other fungi decreased in prevalence toward healthy tissue. *S. rolfsii* and *Geotrichum* sp. were not found in healthy stolons. *Rhizoctonia* sp., *Choanephora* sp., *Curvularia* spp., and *Sclerotium bataticola* were isolated occasionally, mostly from stolons that appeared to be healthy. *Trichoderma* sp. and *Geotrichum* sp.

were strongly antagonistic to *S. rolfsii*. The other fungi exhibited various degrees of inhibitory and stimulatory effects upon *S. rolfsii* and upon one another.

*Resistance to root-knot nematodes in cowpea.* HARE, W. W. Iron cowpea and 4 breeding lines (M255, M455, M755, and M855) were rated resistant to the root-knot nematodes *Meloidogyne incognita*, *M. incognita acrita*, *M. javanica*, and *M. arenaria* when young plants were tested in the greenhouse. Arlington, Groit, Red Chinese, Long Pod Cream, Extra Early Blackeye, Bunch Purple Hull, Mississippi Crowder, Dixilee, and Brown Sugar Crowder were susceptible. There were no galls on the resistant varieties after inoculation with *M. arenaria*, but small galls were produced by the other 3 nematodes. Eighty $F_3$ progenies of M455 × Brown Sugar Crowder and the parental varieties were inoculated with *M. incognita*. Eighteen progenies were resistant, 38 were segregating, and 24 were susceptible. In the segregating progenies, individual plants were approximately 2.3 resistant to 1 susceptible. The results suggest that resistance to *M. incognita* is controlled by a single gene pair with resistance dominant to susceptibility.

*Control of tobacco mosaic virus (TMV) with milk.* HARE, W. W., and G. B. LUCAS. California Wonder pepper plants in the greenhouse were sprayed with Grade A or powdered cow's milk. Plants were then rubbed with fingers dipped in TMV solution a) immediately, b) 24, or c) 48 hours later. In 14–25 days the % plants with mosaic was a) 10, b) 10, and c) 20, whereas 92% of unsprayed inoculated plants had symptoms. Dipping fingers in TMV, then milk, before rubbing plants also controlled the disease. Similar results were obtained with greenhouse tomatoes and tobacco transplanted to the field. Little control resulted if plants were inoculated with TMV and then sprayed with milk. The possible role and value of milk or its by-products in control of both plant and animal viruses has wide implications.

*Tests with Terraclor in 1958 for the control of southern blight of Spanish peanuts.* HARRISON, A. L. Terraclor (pentachloronitrobenzene) applied as a 10% dust in a 12-in. band over the seed row at planting time reduced losses from southern blight (*Sclerotium rolfsii*) by increasing yields and grade of sound mature kernels (SMK) in commercial tests in South Texas. In one large replicated commercial test at Pearsall, where 10% Terraclor was applied at 100–200 lb./A., the average yield was 2062 lb. of peanuts per acre with a grade of 64% SMK for the treated areas and 1394 lb. of peanuts per acre with a grade of 56% SMK for the adjoining untreated areas. In small replicated randomized plots, Terraclor at 10–20 lb./A. of active fungicide increased yields and grade of peanuts when applied as a spray or dust to the crown of peanut plants or as a dust in a 12-in. band over the seed row at planting time. A single application of Terraclor was as effective as split applications in controlling southern blight in these tests. The results at rates below 10 lb./A. were inconsistent.

*Histological studies of roots of pine seedlings infected with black root rot.* HODGES, CHARLES S. One of the characteristic symptoms of black root rot of pine seedlings is enlarged black roughened areas on the tap root and larger laterals. A histological study of these areas showed that the cortex was greatly proliferated, due to increased activity of the phellogen and multiplication of the cortex cells. The cortex of the diseased roots varied from 10 to 30 layers of rounded cells, many showing evidence of both periclinal and anticlinal division. In contrast, the cortex of a normal pine root consisted of 2–3 layers of elongated cells, which normally have no meristematic activity. The periderm of the diseased roots was also thicker than in normal roots. No mycelium was found in the cortex, although it was commonly observed in the periderm. The abnormal cell divisions indicated a response to some growth-regulatory substance. The absence of mycelium in the cortex itself suggested that such a substance may be produced by fungi occurring in the periderm or rhizosphere. *Sclerotium bataticola* and *Fusarium* spp. were found to be constantly associated with the disease and to be pathogenic in inoculation tests. Assay of these fungi for auxin production showed that *S. bataticola* produced indoleacetic acid, whereas the isolates of *Fusarium* tested did not. Addition of as little as 0.1 ppm indoleacetic acid to the nutrient solution in which pine seedlings were growing stimulated cell division in the roots.

*Studies on Helminthosporium leaf blotch of oats (Pyrenophora avenae).* IVANOFF, S. S. Leaf blotch has been a serious disease on oats in Mississippi during the past 3 years. It was definitely established that, in addition to affecting all aboveground parts, as already known, the causal organism may be an aggressive root parasite of seedlings. Root infection was induced from pathogen-bearing seeds and by artificial seed inoculation in the laboratory in Petri dishes and in ragdolls at 100% relative humidity and 24° and 28°C. Under these conditions, root and coleoptile destruction was accomplished in 12 days. The fungus developed on the outside along the length of radicles, forming arborescent tufts. Soaking heavily contaminated seed lots 24 hours in NaCL (5%) and plain water, and 10 minutes in HgCL (1:1000) reduced seedling infection significantly, but not completely. Control was complete when seed samples producing 66% natural seedling infection were dusted with Ceresan M, ½ oz. per bushel. Of 2000 seeds thus treated in 4 replicated trials, none developed disease symptoms on either roots, coleoptile, or primary leaf. Under field conditions, flower and seed contamination increased during humid periods. Rains after ripening caused great increase in seed disease hazard, hence the importance of timely harvest.

*Cone rust on slash pine controlled by ferbam.* LIGHTLE, PAUL C. A single spray application of ferbam (2 lb. per 100 gal. water) was applied to slash pine flowers to test its effectiveness in controlling cone rust caused by *Cronartium strobilinum* (Arth.) Hedgc. & Hahn. The flowers were in 4 developmental stages: 1) strobilus tip just discernible in the bud scales; 2) strobilus fully exposed but the scales not open; 3) strobilus scales open and horizontal, receptive to pollen; and 4) strobilus scales closed, now a conelet. Two weeks after treatment no phytotoxicity was evident. About 10 weeks after treatment, at the time when the rust was fruiting, infection was determined to have been reduced from 70% for unsprayed controls interspersed among the sprayed trees to 53% for flowers treated in stage 1; to 39% for flowers in stage 2; to 12% for those in stage 3; and 7.5% for flowers sprayed in stage 4. Because flowers on a given tree mature at different times, at least 2 sprays will probably be necessary for adequate protection. It is essential that the number and viability of seeds in sprayed and unsprayed cones be determined before the apparent nontoxicity of ferbam to conelets can be accepted as true.

*Dominant and modifying genes for resistance to black shank of tobacco.* MOORE, E. L. and N. T. POWELL. Resistance to black shank caused by *Phytophthora parasitica* var. *nicotianae* in flue-cured tobacco varieties comes from cigar-wrapper variety Florida 301, and has been regarded as complex and recessive. Susceptible varieties have often been used as recurrent parents in the development of resistant varieties. Lines 5346 and 6047 were selected in the $F_2$ from black-shank-resistant Dixie Bright 102 × susceptible Bottom Special × susceptible Bel 1-76. When these lines were used in crosses, resistance seemed partially dominant and influenced by choice of susceptible parent. From 25 to 80% of the $F_1$ plants survived in critical disease tests. Backcross and $F_2$ generations indicated dominance and the presence of modifying genes in susceptible varieties used as parents. Per cent of diseased plants in backcrosses involving different susceptible varieties was as follows: Warne, 70; Yellow Special A, 79; Virginia 21, 98. Similarly, 37 and 83%, respectively, of $F_2$ plants were diseased from

Hicks × 6047 and Virginia 21 × 6047. Certain $F_1$ and $F_2$ populations suggested that 5346 contained one or more genes for resistance that were not present in 6047. A different source of black shank resistance, line 1002, out of a Santo Domingo cigar wrapper variety, Quin Diaz, acted similarly.

*The genetics of sexual reproduction in Cochliobolus heterostrophus.* NELSON, R. R. A number of genetic factors that block sexual reproduction have been found in *Cochliobolus heterostrophus*. Mutant gene, i, blocks formation of protoperithecia in pairings of compatible isolates, when both carry the gene. The inhibitor gene has been found only in wild-type "a" strains, although its inheritance is independent of the compatibility locus. Certain monoascosporic isolates produce normal-appearing sterile perithecia when selfed or paired with strains of either compatibility group. Ascogenous hyphae fail to develop within these perithecia, indicating that plasmogamy is inhibited. The production of sterile asci by other strains, selfed or paired, is controlled by 2 additional factors that apparently prevent normal reduction division. A cross of an albino strain and an "i" strain produced monoascosporic isolates that are self-sterile, but mate with both parental strains. These isolates cross with all *A* strains, but, of 30 *a* isolates tested, they mate only with the original *a* parent. These results suggest that the compatibility locus may comprise a number of closely linked genes. These studies demonstrate that isolates of the same compatibility group are not genetically similar at all loci involved in the sexual process.

*Further studies on the parasitic weed, Striga asiatica.* NELSON, R. R., and W. E. ROGERS. Recent studies on witchweed, *Striga asiatica*, have been concerned with 1) extending knowledge of the host range of the parasite, 2) evaluating non-gramineous plant species for their ability to induce germination of witchweed seed (i.e., as trap crops), and 3) studying the genetic variability of the parasite. To date, 67 species in 28 genera of the *Gramineae* have been shown to be hosts. About 75% of all grass species tested were parasitized. All species, with a few exceptions, within any tribe of the *Gramineae* were either resistant or susceptible. Thirteen hitherto unreported species in the *Leguminoseae* induced seed germination, although none of them were parasitized. Several investigations indicate possible genetic variability in the parasitic capabilities of witchweed. The strain of witchweed present in North Carolina did not attack any of the 5 non-gramineous species tested, which previously were reported as hosts. Five selections of sorghum, considered immune to witchweed in South Africa, were parasitized. The formation of well-developed appressoria on roots of soybeans indicates parasitic variability. The finding of yellow-flowered witchweed in populations of normal red-flowered plants also indicates genetic diversity.

*The effect of infrared irradiation on the incidence of tobacco ringspot virus disease symptoms in watermelon.* ROSBERG, DAVID W. Infrared irradiation of watermelon seed harvested from "pimpled" watermelon fruits infected with the tobacco ringspot virus greatly reduced the incidence of pimples symptoms in field-grown watermelons. The incidence of pimpled fruits is normally 25–33% when seed from an infected fruit are planted. The seeds were irradiated at temperatures of 70, 75, 80, 85 and 90°C for 5–30 minutes, totaling 18 different treatments. The incidence of pimples disease symptoms in irradiated seed plots was less than 2.0%, compared to 37.8% in the control plots. Infrared irradiation of watermelon seed markedly retards germination and growth of the young seedlings, delaying fruit maturation about 10–14 days. It is not as yet clear whether infrared irradiation inactivates the tobacco ringspot virus in the seed or masks the symptoms.

*Influence of resistance to Heterodera glycines on soybean yield and nematode populations.* Ross, J. P. The soybean cyst nema does not reproduce on the roots of the Peking variety of soybean. For determination of the influence of this resistance on soybean yields and nema populations, Peking and a susceptible soybean line of similar maturity were planted in treated and untreated infested soil. Granular Nemagon, broadcast at 3 gal. of fumigant per acre, was used to treat a strip of infested soil 18 × 280 ft. 9 days before planting. The contiguous strips of treated and untreated soil were divided into 6 paired blocks, and the same randomization plan was used for arranging 18-ft. plots in both soils. Respective yields of susceptible and resistant plants growing in unfumigated plots were 37 and 66% of the yields, of the same soybean lines growing in fumigated plots. In unfumigated soil 1 month after planting, root nodulation was abundant on Peking and extremely sparse on the susceptible line. Nema populations in unfumigated plots, 2½ weeks after planting, were correlated ($r = -0.8206$) with soybean yield of the susceptible line, while there was no correlation for Peking. After maturity, the average nema population in unfumigated plots was 3 (range, 0–10) larvae per pint of soil for Peking and 320 (range, 20–1100) for the susceptible line.

*The influence of root-knot-nematode-infected seed pieces and infested soil on potato production.* SASSER, J. N., and FOIL W. MCLAUGHLIN. The influence of root-knot nematode (*Meloidogyne incognita*) infected seed pieces and infested soil on yield and quality of potatoes was investigated in a split-plot design as follows: Treated (D-D, 1,2-dichloropropene-dichloropropane broadcast 20 gal./A.) and untreated soil were planted with seed pieces from 1) infected tubers, 2) symptomless tubers selected from an infected crop, and 3) certified tubers. Single-row plots 25 ft. long were replicated 4 times. Average total yields (cwt. U.S. No. 1 and No. 2 size potatoes) from the 3 types of seed pieces in treated and untreated soil were: 1) 62.1 vs 64.6; 2) 91.9 vs 74.6; and 3) 114.3 vs 84.5. When badly infected tubers were removed, the comparative yields of marketable potatoes from the 3 types of seed pieces in treated and untreated soils were: 1) 37.3 vs 22.0; 2) 87.3 vs 12.7; and 3) 112.0 vs 27.0. Seed quality was of little importance when potatoes were planted in infested soil. Selected as well as obviously infected tubers increased inoculum in the soil and decreased yields and quality.

*Irish potato seed-piece treatment with potassium gibberellate.* SINCLAIR, J. B. Potato seed pieces of the varieties Triumph and Red La Soda were dusted with 3 concentrations of potassium gibberellate (25, 50, and 100 ppm) and planted in the field in a randomized block design with 4 replications. There were 30 seed pieces for each treatment, including an untreated plot. A stand count showed a tendency for a decrease in stand below that of the untreated plot, as the concentration of potassium gibberellate was increased, but the differences were not significant. Yield data revealed significant decreases, when compared to the untreated plot, in pounds of marketable tubers, as well as the total pounds of tubers produced in both varieties as the concentration of potassium gibberellate was increased. There was a tendency for an increase in pounds of culls produced above the untreated plot, as the concentration of potassium gibberellate was increased, but the differences were not significant.

*Factors influencing emergence of larvae from cysts of Heterodera glycines Ichinohe. Influence of constant temperature.* SLACK, D. A., and M. L. HAMBLEN. In replicated and repeated tests, lots of 50 selected cysts were incubated in a small quantity of water in syracuse watch glasses at intervals of 4°C from freezing to 44°C. Of 650 cysts incubated at each temperature, emergence of larvae from cysts was not observed at temperatures below 16°C or above 36°. Average larval emergence from each 50-cyst lot increased with each increase in temperature, from 77 larvae

at 16°C to 1143 larvae at 24°C, and then decreased with each increase in temperature, to 39 larvae at 36°C. Transfer to 24°C of cysts incubated for 1 month at 12°C or below resulted in larval emergence; however, total emergence was reduced greatly below that of cyst lots incubated originally at 24°C. Incubation of cysts below 12°C for 1 week or less caused no reduction in total emergence of larvae when placed at 24°C. Cysts incubated at 40°C for 1 week and then placed at 24°C resulted in larval emergence of less than 1% of comparable cysts incubated at 24°C. Larvae failed to emerge from cysts incubated at 40° C for 10 days or longer when placed at 24°C. Emerged larvae were killed by freezing and by incubation at 40°C for 3 hours. Some emerged larvae, however, persisted for periods exceeding 30 days when incubated at temperatures between 4°C and 28°C.

*Growth rates and inoculation methods for a Helminthosporium inciting oat chlorosis.* THOMAS, M. H., JR., and R. W. EARHART. Growth studies under controlled temperature and light conditions on a *Helminthosporium* species capable of causing oat chlorosis indicated optimum growth at 28°C on 5 different culture media. Potato-dextrose and Lima-bean agar produced the fastest growth. Optimal temperature was the same for either light or dark growing conditions, but growth was faster in the light, and spore production greater in the dark. Symptoms were induced on greenhouse-grown plants of Victorgrain 48-93 and Arlington by 3 different inoculation methods: 1) artificially infesting seeds; 2) artificially infesting soil; and 3) spraying aqueous suspensions of the fungus cultures on foliage. The artificial infesting of seed and soil has not always been successful on first planting; however, replanting in the previously infested soil produced typical symptoms. This probably indicates a necessity for the organism to build up to pathogenic levels in the soil. Foliage inoculation produced foliar lesions similar to, but not identical with, those produced by *H. victoriae*, or *H. sativum*; these frequently resulted in complete necrosis of the infected leaf blade.

*Relation of host nutrition to the development of bacterial wilt of alfalfa.* WALTERS, H. J., and E. M. CRALLEY. The effects of host mineral nutrition on development of bacterial wilt of alfalfa (*Corynebacterium insidiosum* (McCull) H. L. Jens.) were studied in sand culture in a greenhouse kept at about 27°C. Three levels of potassium—low (5 ppm), medium (25 ppm), and high (50 ppm)—were used in nutrient solutions in combination with low (25 ppm) and high (50 ppm) nitrogen, and low (7.5 ppm) and high (30 ppm) phosphorous. The levels of calcium, magnesium, and trace elements were held constant during the experiment. Three tests conducted over 3 years show that the susceptibility to bacterial wilt of Buffalo and Grimm varieties of alfalfa is definitely related to the level of potassium. Plant growth was poorest and bacterial wilt was most severe when the levels of nitrogen and phosphorous were high and the level of potassium low. Bacterial wilt infection was lowest when the levels of nitrogen and phosphorous were low and the level of potassium medium or high.

*Studies on a Phytophthora isolate causing seed piece rot of sugarcane in Louisiana.* VAN DER ZWET, T. In the spring of 1948 a seed piece rot of sugarcane was found in Louisiana, and 3 different *Phytophthora*-like cultures were isolated. Pathogenicity tests indicated that only 2 of these would cause a severe rot of the seed pieces, one of which was identified as *P. erythroseptica*. The other isolate, being the more virulent one, could not be identified at that time, because sporangia and sexual bodies were absent. Present studies, however, have shown that this isolate will produce fruiting structures on sterilized oat grains. When these grains were placed in tap water, many sporangia and chlamydospores were produced within 48 hours. The sporangia, borne on long sporangiophores, have inconspicuous papillae, and vary in shape from ovoid to bluntly ellipsoid. They germinate after several hours and produce many zoospores, which encyst after a short swarming period. Secondary sporangia are frequently produced by successive proliferation through the empty ones. Chlamydospores are very common, intercalary and spherical in size. They germinate by 1–7 germtubes. The antheridia are amphygenously attached to the oogonia, while the oospores are plerotic and have a thick epispore. The isolate has an optimum growth temperature of 22°C, with a range of 6–32°C. Comparative studies are in progress for definite identification of this isolate.

# PHYTOPATHOLOGICAL NOTES

*Reversal of Captan Fungitoxicity by l-histidine.* SAUL RICH. Lukens and Sisler[1] showed that the toxicity of captan[2] to yeast can be reversed by sulfhydryl-containing compounds such as cysteine, homocysteine, and glutathione. There is no reversal if captan is in contact with yeast cells for more than 1 hour before the sulfhydryl compounds are added. These compounds react quantitatively and rapidly to destroy captan. The reversal of the toxicity of captan by sulfhydryl compounds is apparently due to destruction of the captan in the ambient fluid.

Because *l*-histidine reverses the toxicity of a number of fungitoxicants, its action on captan was studied, using mycelial growth of *Monilinia fructicola* as the criterion of toxicity. In these studies the fungus was grown on a liquid medium containing the following, in grams per liter: 2.0 $KNO_3$, 15.0 dextrose, 1.5 $KH_2PO_4$, 1.0 $MgSO_4$, 1.0 yeast extract, and 10.0 Hoagland's A-Z solution. The pH of the autoclaved complete nutrient was 5.5. The captan, in acetone solution, and the reversing amino acids were added only after the flasks plus nutrient were autoclaved and cooled. The inoculum was a water suspension of mycelial fragments and conidia. Equal volumes of inoculum were used for each flask. Captan was added just before the inoculum. Growth was recorded after 4–7 days.

Captan at $3\times10^{-5}M$ completely inhibited the growth of the fungus. This toxicity was reversed by $10^{-2}M$ *l*-histidine and $10^{-2}M$ *l*-cysteine. In time-course experiments (Table 1), *l*-histidine could reverse toxicity if added 24 hours after the addition of captan; *l*-cysteine could reverse toxicity if added up to 6 hours after captan, but not after 24 hours.

There was no proportionality between the amount of captan added and the amount of *l*-histidine needed for reversal. Captan, then, is apparently not a competitive inhibitor of *l*-histidine.

Because *l*-histidine can reverse the toxicity of captan long after the time when captan-destroying *l*-cysteine ceases to be effective, *l*-histidine must reverse the toxic effects of captan within the cell.

Lukens[3] was not able to reverse the toxicity of captan to yeast cells by adding histidine. This indicated that the vital sites susceptible to captan poisoning differ in yeast from those in *M. fructicola*. Lukens and Sisler proposed that captan is a non-specific poison that destroys sulfhydryl groups or releases toxic products within the cell. If this is so, then *M. fructicola* is able to shunt every system poisoned by captan except those essential to the synthesis or utilization of histidine.—The Connecticut Agricultural Experiment Station, New Haven.

TABLE 1.—*Time-course study of reversal of the fungitoxicity of $3\times10^{-5}M$ captan by $10^{-2}M$ l-histidine and $10^{-2}M$ l-cysteine*

| Hours between adding captan and adding amino acids | Mycelial growth[a] | |
|---|---|---|
| | Histidine | Cysteine |
| 0 | + | + |
| 1 | + | + |
| 3 | + | + |
| 6 | + | + |
| 24 | + | — |

[a] + indicates growth of *Monilinia fructicola*; — indicates no growth.

*A Caffeine Additive to Aid Mechanical Transmission of Necrotic Ring Spot Virus from Fruit Trees to Cucumber.*[1] T. O. DIENER AND M. L. WEAVER. Necrotic ring spot virus[2] is mechanically transmissible from infected cherry and peach leaves to cucumber,[3] but transmission is possible only during the spring months. Even then, rates of transmission are often low with some virus strains and with inoculum from certain *Prunus* species. These limitations affect the usefulness of cucumber as an indicator host for necrotic ring spot virus.

In attempts to improve the rate of transmission, infected leaves were ground in solutions containing various additives. Several alkaloids were included in the series. Of the compounds tested, caffeine had by far the greatest effect. The results achieved with this alkaloid are summarized here.

Inoculum was secured from *Prunus avium*, *P. cerasus*, and *P. mahaleb* trees in the field and from *P. tomentosa* seedlings in the greenhouse. In each experiment, the leaves were divided at random into 2 lots of equal weight. One lot was ground in a mortar with 2 volumes of either water or 0.01*M* phosphate buffer (pH 7.5), the other lot with 2 volumes of an aqueous caffeine solution. The resulting suspensions were expressed through cheesecloth. Inoculations were made by lightly rubbing cucumber cotyledons, previously dusted with carborundum (400 mesh), using a pad of cheesecloth moistened with the inoculum. Each suspension was inoculated to 20–30 green-

[1] Lukens, R. J., and H. D. Sisler. 1957. Studies on the chemistry of the fungitoxicity of captan. Phytopathology 47: 22-23.

[2] *N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide.

[3] Lukens, R. J. 1957. Chemical reactions involved in the fungitoxicity of captan. Ph.D. thesis. University of Maryland, College Park, Md. 51 p.

[1] Scientific Paper No. 1775, Washington Agricultural Experiment Stations, Pullman. Project No. 1411.

[2] Berkeley, G. H., D. Cation, E. M. Hildebrand, G. W. Keitt, and J. D. Moore. 1951. Necrotic ring spot. *In* Virus diseases and other disorders with virus-like symptoms of stone fruits in North America. U. S. Dept. Agr., Agr. Handbook 10, 164-170.

[3] Moore, J. D., J. S. Boyle, and G. W. Keitt. 1948. Mechanical transmission of a virus disease to cucumber from sour cherry. Science 108: 623-624.

house grown cucumber plants (var. National Pickling) at the stage when the first true leaf was emerging.

Preliminary trials showed that solutions containing 1% or more of caffeine (w/v) caused visible damage when applied to cucumber cotyledons. No damage occurred with solutions containing 0.5% or less of caffeine. Therefore, all tests were made with 0.5% solutions of caffeine.

Transmission tests from *P. avium* trees were started shortly after bud-break, and continued through July. Typical results with leaves from the variety Lambert are shown in Table 1. The results were essentially identical in transmission tests from varieties Bing and Royal Ann. In all tests, rates of transmission were substantially higher with caffeine solutions than with water or phosphate buffer. Using caffeine, the virus could be transmitted from sweet cherry leaves into cucumbers 3–4 weeks after such transfers had become impossible with water or buffer alone. However, tests in July were negative with all solutions.

Caffeine also increased the longevity of the virus *in vitro*. Inoculum in water or buffer lost infectivity after storage for 3.0–6.5 hours at 10°C, whereas inoculum in caffeine solution consistently retained some infectivity after 22 hours at 10°C.

The results of transmission tests from *P. cerasus* (var. Montmorency) leaves were similar to those from sweet cherry leaves. Again, transmission rates and longevity *in vitro* were increased when caffeine was present in the inoculum.

No such effects were detected in transmission tests from either *P. mahaleb* leaves or cucumber leaves to cucumber. However, in both cases, transmission rates were regularly high without the addition of caffeine.

To the authors' knowledge, transmission tests from *P. tomentosa* to cucumber have always been unsuccessful.[4] However, when transmission from this host was attempted with caffeine in 4 tests in April, 4–14% of the inoculated cucumber plants developed symptoms. All plants inoculated with suspensions of *P. tomentosa* leaves in water or phosphate buffer remained healthy.

The results indicate that adding caffeine to the suspending medium has the following advantages: 1) The higher rate of transmission from sweet and sour cherry leaves increases accuracy in indexing and requires fewer cucumber plants. 2) Transmission from sweet and sour cherry leaves is possible for longer in the spring. 3) Increased longevity of the virus *in vitro* eliminates a need for immediate inoculation (inoculum from sweet and sour cherry leaves can be stored 2–3 hours in the refrigerator before inoculation). 4) Transmission is possible from *P. tomentosa* leaves to cucumber.—Irrigation Experiment Station, Prosser, Washington.

TABLE 1.—*The effect of caffeine on transmission of necrotic ring spot virus from leaves of Prunus avium (var. Lambert) to cucumber*

| Inoculum storage at 10°C (hours) | Infectivity of inoculum[a] | | |
|---|---|---|---|
| | In water | In 0.01*M* phosphate buffer | In 0.5% caffeine solution |
| Test on April 8 | | | |
| 0 | 13 | 16 | 94 |
| 3.5 | 0 | 4 | 73 |
| 6.5 | 0 | 0 | 31 |
| 22 | 0 | 0 | 6 |
| Test on May 1 | | | |
| 0 | 0 | 7 | 27 |
| 3.5 | 0 | 4 | 20 |
| 6.5 | 0 | 6 | 44 |
| 22 | 0 | 0 | 13 |

[a] Percentage of inoculated plants developing symptoms.

## BOOK LISTING

GILL, N. T., and K. C. VEAR. 1958. **Agricultural botany.** Gerald Duckworth & Co., London. 636 p. Price $12.00. (Distributed by The Macmillan Co., New York)

The authors of this textbook, published in England September 2, 1958, are, respectively, Head of the Biology Department, Harper Adams Agricultural College, Newport, Salop, and Head of the Botany Department, Seale Hayne Agricultural College, Newton Abbot, Devon. Although designed primarily for British students, and dealing largely with the crops grown in Great Britain, it contains information of value to any beginning agricultural student. The book is divided into four sections as follows: 1) Plant breeding and crop improvement, 2) Botany of crop plants, 3) Farm weeds, and 4) Diseases of farm crops. Although some acquaintance with botany is assumed, the book is written for students with little or no knowledge of the subject. A glossary of botanical terms and an appendix giving the derivation of generic and specific names are included.

[4] Gilmer, R. M. 1956. Behavior of juice extracts of certain virus isolates from prunus and from rose in cucumber plants. (Abstr.) Phytopathology 46: 241.